Mediterranean diet, cardiovascular health and longevity: Strategies to improve the assessment and interpretation of this dietary pattern in nutritional epidemiology

Tognon, G.

Mediterranean diet, cardiovascular health and longevity

Strategies to improve the assessment and interpretation of this dietary pattern in nutritional epidemiology

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Abstract

Introduction - Adherence to the Mediterranean diet, could reduce the risk of mortality and CVD across the life course. However, its epidemiological instrumentation needs to be contextualized and adapted to local cultures. Moreover, there is evidence that typical dairy products should be disaggregated in the MDS due to divergent health properties.

Methods - The Västerbotten Intervention Program (VIP), the Northern Sweden MONICA, the H70 elderly study, the Copenhagen MONICA study, and the IDEFICS EU children study were included. A refined version of the Mediterranean Diet Score (MDS) was calculated from diet histories (H70), FFQs (VIP), and food records (MONICA-Copenhagen) by including foods that better describe the context of a genuine Mediterranean diet (e.g., wholegrain instead of total cereals) and ingredients from mixed dishes. Cox models, adjusted for potential confounders, were used to compare the original and refined MDS and their associations with mortality, CVD incidence, and mortality. Adjusted Cox models were also used to test the association between dairy intakes and the risk of mortality in the VIP and H70 cohorts. Adherence among children was assessed using both FFQ and recall data, and its association with BMI, waist circumference, WtHR, and % fat mass was assessed cross-sectionally and longitudinally.

Results – The refined MDS, but not the original score, inversely predicted the risk of all-cause mortality in the MONICA-Copenhagen (HR = 0.93) and VIP (HR = 0.95) studies, as well as with CVD incidence and CVD mortality in all cohort studies. Stroke incidence and stroke mortality were not associated with both scores. Milk intakes directly predicted all-cause mortality (HR = 1.02, 95% CI: 1.00, 1.05) whereas cheese intakes showed an inverse association with all-cause mortality (HR = 0.94, 95% CI: 0.91, 0.97). Fermented milk intakes

showed a borderline significant inverse association with all-cause mortality. The MDS was inversely associated with the risk of overweight and obesity in children with low prevalence of high-adherent children in all countries (< 30%) except Sweden.

Discussion and conclusions - The adoption of a Mediterranean-like diet is protective for CVD across the lifespan, and adherence should be increased among children. The sensitivity of assessments evaluating adherence to this pattern can be increased. The results on dairy product intakes suggest differences between milk and cheese, but they should be interpreted cautiously.

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The Mediterranean diet score and mortality are inversely associated in adults living in the subarctic region.
Tognon G, Nilsson LM, Lissner L, Johansson I, Hallmans G, Lindahl B, Winkvist A. J *Nutr.* 2012;142(8):1547-53.

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This thesis analysed data from several cohorts and research studies, and I had the privilege to collaborate with prominent scientists from all over Europe. I would like to thank them for their work and the support I received: Ingegerd Johansson, Elisabeth Rothenberg, Berit Heitmann, Wolfgang Åhrens, Vittorio Krogh, and Alfonso Siani as well as their co-workers. Thanks for allowing me to work on so much data you and your staff have been collecting for many years. And, of course, a gigantic “thank you” goes to both Valter Sundh and Kirsten Mehlig; your sound competence in statistics contributed to a series of reliable scientific results and many publications.

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and, most of all, my family in Italy who always encouraged me to get a degree and become an educated person. Thanks everyone, you are the main reasons I keep challenging myself to become more knowledgeable in nutrition and turn my passion into something useful for our society.

Author's declaration

I declare that all the material contained in this thesis is my own work. The analyses included in papers cited were checked by collaborators prior to publication. Those related to the manuscript on the Mediterranean diet in the Västerbotten Intervention Programme (VIP) (Tognon *et al.,* 2012) were checked by Lena Nillsson. Ingegerd Johansson and Dmitri Shungin performed some analyses in one of the two manuscripts on dairy products and mortality (Tognon *et al.,* 2017, 2018). More details about the authors who performed the statistical analyse are reported below. The co-authors on all manuscripts included in this thesis contributed to preparation of a suitable dataset, and provided statistical support and advice during the writing of the manuscript. All details included within this submission is my own work.

Manuscript	**Analyses performed by**
Tognon *et al.,* 2012. The Mediterranean diet score and mortality are inversely associated in adults living in the subarctic region.	I performed all analyses and Lena Nilsson repeated them to confirm the results.
Tognon *et al.,* 2014b. The Mediterranean diet in relation to mortality and CVD: a Danish cohort study.	I performed all analyses.
Tognon *et al.,* 2011. Does the Mediterranean diet predict longevity in the elderly? A Swedish perspective.	
Tognon *et al.,* 2017. Non-fermented milk and other dairy products: associations with all-cause mortality.	Ingegerd Johansson performed the analyses reported in tables 5 and 6, Dmitri Shungin performed the analyses on Mendelian randomization. I performed all the remaining analyses.
Tognon *et al.,* 2018. Dairy product intake and mortality in a cohort of 70-year-old Swedes: a contribution to the Nordic diet discussion.	I performed all analyses.
Tognon *et al.,* 2014a. Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study.	

Tognon *et al.,* 2014c. Adherence to a Mediterranean-like dietary pattern in children from eight European countries. The IDEFICS study.

Gianluca Tognon

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List of abbreviations

24HDR	24-hour diet recall
BMI	Body Mass Index
BMR	Basal Metabolic Rate
CEHQ-FFQ	Children's Eating Habits Questionnaire-Food Frequency Questionnaire
CHD	Coronary Heart Disease
CIs	Confidence Intervals
CV	Cardiovascular
CVD	Cardiovascular Disease
DLW	Doubly-Labelled Water
EU	European Union
FIL	Food Intake Level
FFQ	Food Frequency Questionnaire
H70	The Gerontological and Geriatric Population Studies in Gothenburg
HR	Hazard Ratio
IDEFICS	Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS
IHD	Ischaemic Heart Disease
fMDS	frequency-based Mediterranean Diet Score
MDS	Mediterranean Diet Score
MFGM	Milk Fat Globule Membrane
MI	Myocardial Infarction
MONICA	Multi-national Monitoring of Trends and Determinants in Cardiovascular Disease
MUFA	Mono-Unsaturated Fatty Acids
NCD	Non-Communicable Disease
OR	Odds Ratio
PAL	Physical Activity Levels
PUFA	Poly-Unsaturated Fatty Acids
SACINA	Self-Administered Children and Infant Nutrition Assessment
SFA	Saturated Fatty Acids
VIP	Västerbotten Intervention Program
WHO	World Health Organization
WtHR	Waist-to-Hip Ratio

1. Background and Rationale

Cardiovascular disease (CVD) is a general term used to identify a group of disorders of heart and blood vessels that can cause both death and disability. In 2015, 49 million people were living with CVD in the European Union (EU). The number of deaths caused by CVD in the EU was approximately 1.8 million, corresponding to 37% of all deaths. In men, CVD represented the main cause of death in all countries except the following 11, where cancer explained more deaths: Belgium, Denmark, France, Italy, Luxembourg, the Netherlands, Norway, Portugal, Slovenia, Spain and the UK. In women, CVD was the main cause of death in all countries except Denmark, where cancer causes the highest number of deaths. CVD causes a loss greater than 26 million DALYs (19% of total DALYs) in the EU (Wilkins *et al.,* 2017).

Figure 1 summarizes death rates across European countries (both EU and non-EU countries) (WHO, 2018). Contrary to what had been observed during the 7-country study in the '60s, Mediterranean countries are not characterized by the lowest CVD mortality rates. On the contrary, some of the former Yugoslavian countries (Former Yugoslavian Republic of Macedonia, Serbia, Montenegro, Bosnia and Herzegovina) appear to have the highest CVD mortality rates in Europe. Notably, although the number of CVD-related deaths have decreased from 259.9 deaths/100,000 subjects in 2007 to 233.1 deaths/100,000 subjects in 2017, the total number of deaths due to CVD increased by 21% during the same period, potentially due to population growth (Roth *et al.,* 2018).

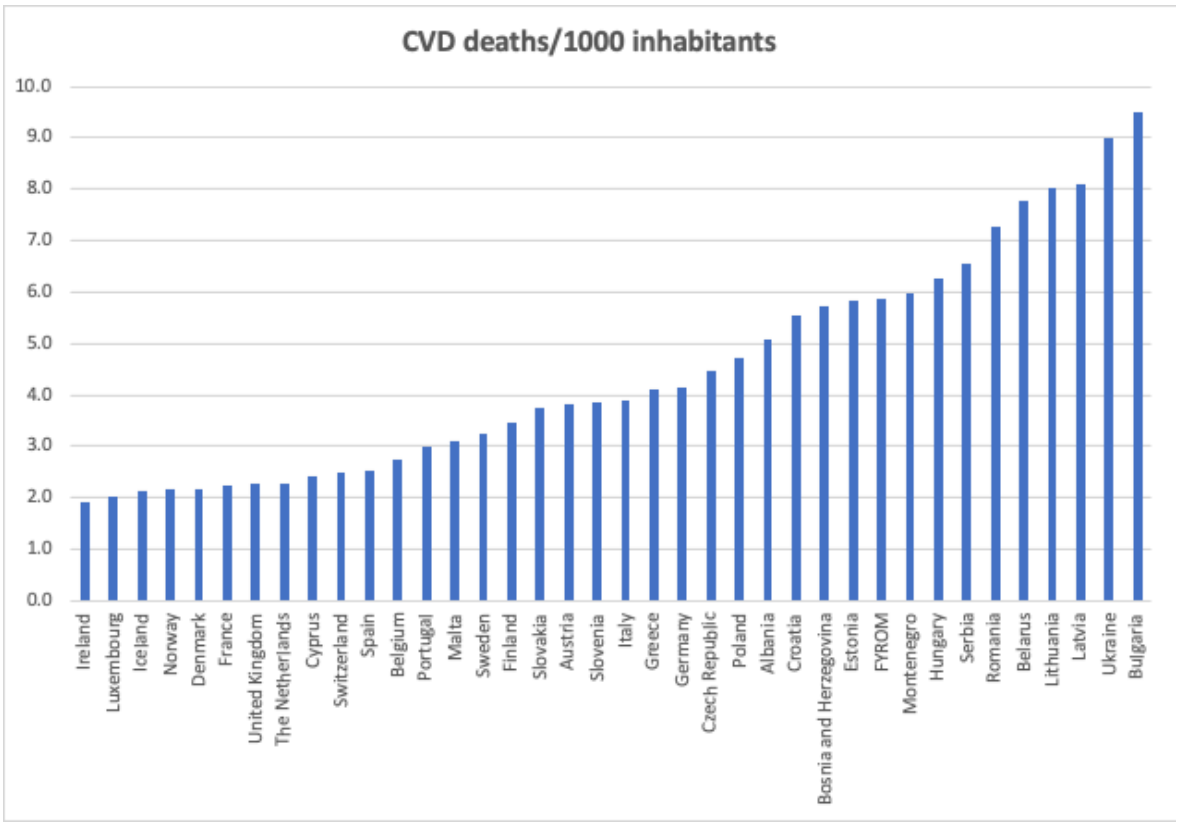

Figure 1: Number of CVD deaths per 1000 inhabitants in European countries
The number of CVD deaths have been calculated based ono data obtained from individual country profiles published by the WHO (2018). Both EU and non-EU countries are included to include all Mediterranean countries. FYROM = Former Yugoslavian Republic of Macedonia.

The health and economic burdens associated with CVD are difficult to quantify accurately and consistently, primarily because of the disparities incurred by socioeconomic development. Consequently, CVD-related costs are escalating. In England alone, they are estimated to be greater than £7 billion per year, while the cost to the national economy (which includes associated loss of productivity) exceeds £ 15 billion (Public Health England, 2019).

With an exception made for the strict anti-smoking policies applied in many countries, and despite CVDs being largely preventable, policy-makers have surprisingly not yet identified the most effective preventive strategies that could sensibly reduce the prevalence of these

diseases. However, a better contextualisation of CVD risk factors would enable a more tailored design of prevention strategies. The latter should be culturally relevant to the population's diet, causes, and socioeconomic outcomes in their design and analysis to extend understanding of how best intervention strategies can target this complex, prevalent disease. Implementation of successful strategies to reduce the prevalence of well-known modifiable risk factors (e.g. tobacco and alcohol use, unhealthy diets and low physical activity), positively impact common CVD mediators such as hypertension, obesity and hypercholesterolaemia (Forouzanfar *et al.,* 2016). A reduction in prevalence of the latter would theoretically lead to the prevention of 18 million deaths annually (Yusuf *et al.,* 2019). Among the lifestyle factors that can impact both CVD and its mediators, diet has become the focus of an exponentially increasing number of studies which started in the early '50s.

To study dietary exposure is complicated and researchers have not yet been able to agree on how best to elucidate what constitutes a healthy diet or, therefore, to quantify the effects of dietary habits on CVD risk (Yu *et al.,* 2018). Unfortunately, the evolution of dietary studies encompasses a series of errors and distortions. The most relevant example, sparked during the '80 and '90s, links to guidelines promoting restriction of total fat intake in favour of carbohydrates, while ignoring the quality and sources of these nutrients (Liu *et al.,* 2017). This concept became a (progressively) predominant dietary recommendation, followed by many people and embraced by the food industry, which started to sell low-fat (but sugar-added) food products (Moss, 2014). Although a causal link has never been proven, and the progressive increase in sedentary behaviours might have played a role as well, this focus was accompanied by a series of unintended consequences including an increase in the

prevalence of obesity, type 2 diabetes and CVD – all of which have subsequently been associated with increased consumption of high-glycaemic index carbohydrates (Zhu *et al.,* 2019). The focus on reducing total fat intake led to the victimisation of full-fat dairy products (particularly cheese), whose consumption has been discouraged for many years despite a clear lack in any evidence in support of such a recommendation (Thorning *et al.,* 2016). Many years passed before the focus of both consumers and official recommendations shifted away from fat and, from individual nutrients to foods. The importance of studying and recommending dietary patterns was eventually acknowledged (Alasfoor *et al.,* 2009). However, the study of dietary patterns is at a very immature stage and future studies are necessary to precisely assess both adherence to specific dietary patterns and to correctly measure their impact on CVD risk.

Risk factors accumulate (and interact) during the entire life course before they (typically) manifest in adulthood. Atherosclerosis, in particular, is now recognised to be a slow, progressive disease that initiates in childhood. The Dutch Hunger-Winter study highlighted the developmental origins of this disease group, evidencing a dose-response association between intrauterine deprivation and metabolic risk in adulthood (Van Abeelen *et al.,* 2012). Notably, the risk of atherosclerosis during adulthood has been directly predicted by the level of total adiposity and truncal subcutaneous fat accumulation during adolescence (Engin, 2017) and by accelerated weight gain during the first years of life (Buffarini *et al.,* 2018). Children only rarely develop CVDs, while childhood obesity is the most relevant early risk factor for future CVD risk (Chung *et al.,* 2018). Results from the Childhood Obesity Surveillance Initiative (COSI) on a representative sample of 250,000 European children, indicated that, in 2010, the prevalence of European children aged six years who were overweight or obese varied substantially within EU countries. The latter spanned from 19.3

among 6-year-old children from Belgium to 49% of 8-year-old Italian children and 57.2% of 9-year-old Greek children (WHO, 2018). These results indicate that large variations among European countries exist, with greater prevalence in South European countries.

A recent systematic review and meta-analysis found that childhood obesity is directly related to higher blood pressure and triglyceride levels (and to lower HDL levels) in later life. Notably, this is independent of Body Mass Index (BMI) during adulthood (Umer *et al.,* 2017). However, no specific factor or point in time is specifically linked to the risk of CVD, since the latter is the result of the accumulation of several risk factors during the life course

A life course approach to the study of dietary patterns is likely to allow a more accurate estimate of the impact on CVD. In particular, the scientific community now acknowledges the importance of exposure to CVD risk factors in early life and their association with later health consequences (Halfon *et al.,* 2017).

2. Cultural adaptation and contextualisation of the Mediterranean diet score

Created by Trichopoulou *et al.* (1995), the Mediterranean diet score (MDS) assesses adherence to a Mediterranean-like diet. The MDS is calculated by giving an increasing number of points to subjects characterized by high intakes of vegetable foods (i.e., grains, legumes, fruit, nuts, seeds, vegetables) and fish. One additional point is allocated for low intakes of meat and another for low intakes of dairy products (particularly fermented milk and cheese). The population median intakes for each of the above food groups (adjusted to 2,000 kcal in women and 2,500 kcal in men) is used as a threshold for both high and low intakes. Encouragingly, this score resonates with a recent systematic review, which found that suboptimal consumption of nuts and seeds, fruit, wholegrain cereals, and vegetables can be a contributing factor for 11 million deaths worldwide. The latter represent one-fifth of global adult fatalities, ten million of which were attributed to CVD (Afshin *et al.,* 2019). However, the MDS does not accurately describe the context of a traditional Mediterranean diet, which is based on high intakes of wholegrain cereals (and not refined grains), moderate intakes of fermented milk and cheese (but low intakes of butter and non-fermented milk), as well as moderate intakes of wine (D'Alessandro and De Pergola, 2018). The MDS is often interpreted as a measure of adherence to a Mediterranean-like diet, although it simply assesses adherence to a generic plant-based, fish-rich diet. This pattern of eating is very similar to other traditional diets including the Healthy Nordic diet (Olsen *et al.,* 2011) and traditional Japanese diets (Sho, 2001), among others. Therefore, the unspecific nature the MDS makes it impossible to quantify the risk reduction imputable to the adherence to a Mediterranean-like diet. This thesis is based on the idea that changes in the MDS calculations, aimed at achieving a more precise description of a Mediterranean-like diet and adaptation of the score to the culture of the population in which it is used,

improves its ability to predict these risks associated with adherence to a diet more in line with the principles of the Mediterranean diet. As an example, in many studies, the distinction between total and wholegrain cereals cannot be made, usually due to the limitations of Food Frequency Questionnaires (FFQs). As a result, consumption of both refined and unrefined grains has often been considered a Mediterranean dietary habit in MDS calculations. Only unrefined grains are typical Mediterranean foods (Aune *et al.,* 2016), and their consumption differs from that of refined grains in terms of CVD risk (Aune *et al.,* 2016). Another common misrepresentation of the context of a Mediterranean-like diet links to consideration of moderate alcohol intakes from any alcoholic drink as a typical Mediterranean habit, while this would only typically include wine, which is also rich in polyphenol compounds, known to reduce CVD risk (Cordova and Sumpio, 2009). The health effects of wine are still controversial, particularly due to associations between alcohol intake and increased risk of some types of cancer (WCRF, 2018). From a CVD perspective, the French Paradox and questions surrounding its validity related to how alcohol impacts CVD risk (Ferrières, 2004), necessitates subtleties in analytic plans to enable the true impact of alcohol intake to be appraised.

Foods are more often consumed in combination (as mixed dishes) than as individual entities. The composition of the latter and the local culture and traditions of each population constitute an indissoluble entity. Every country and, in some cases, every region of the world has its own typical foods and recipes, which reflect a combination of the ingredients available locally and local cooking styles. The studies that have investigated the association between the MDS, chronic disease and mortality, which have been summarized by three meta-analyses (Sofi *et al.,* 2008, 2010, 2014), have often not made clear how

investigators handled mixed foods in the MDS calculations. This step is crucial to understand whether investigators have adapted the score to the culture of each study population. The inclusion of ingredients from mixed dishes in the MDS calculations would, therefore, enable a more precise appraisal of adherence to a Mediterranean-like diet and, consequently, a more precise estimate of its association with CVD risk.

Dairy products, considered atypical Mediterranean foods in the MDS calculations, deserve special considerations since cheese and yoghurt are both typical in a Mediterranean diet. Greek and Turkish yoghurts, Italian and French cheeses and crème fraîche, are all examples of typical Mediterranean dairy products. No evidence to date links consumption of these foods to a higher CVD risk (Guo *et al.,* 2017) therefore the choice of scoring typical Mediterranean dairy products in the calculation of the MDS should be reconsidered. This is especially pertinent given recent evidence showing that the matrix[1] of each specific food modifies many nutrients' health effects (Givens, 2017). The food matrix influences both the bioavailability and rate of absorption of several nutrients and can also affect food digestibility. Milk pasteurisation, homogenisation, and other production methods can negatively affect dairy product's food matrix and health properties. The dairy matrix has been shown to reduce the negative health effects typically associated with saturated fatty acids (SFA) (Fardet *et al.,* 2019) and this could explain why higher SFA intakes do not necessarily correlate with an increased risk of CHD or CVD (De Souza *et al.,* 2015), or why cheese has a weaker effect on blood cholesterol than butter (Biong *et al.,* 2004). Therefore,

[1] The food matrix can be described as the physical domain where all the ingredients and nutrients of a specific food are contained and interact with each other, contributing to characteristics and functionality in the final food as consumed, and that cannot be obtained from the separate ingredients or nutrients (Aguilera, 2018).

it is reasonable to assume that cheese, yoghurt, butter and milk consumption have different, if not opposing, health effects thanks to the fact that their specific food matrix is able to modulate the effects of the nutrients these foods contain (e.g., to mitigate the negative health effect of SFA in some dairy products such as cheese or fermented milk) (Feeney *et al.,* 2018).

In summary, a renewed approach to CVD prevention through a healthy diet should investigate exposure to culturally-adapted and properly contextualised epidemiological representations of dietary patterns instead of individual foods or nutrients. This was done in this thesis by including local recipes and mixed dishes in the MDS calculation (cultural adaptation) and by using intakes of foods that are typical of a traditional Mediterranean diet (e.g., wholegrain cereals instead of total cereals). The study of the different health effects of dairy products constitutes a new exciting area of research that can potentially help understand whether dairy products with similar nutrient composition, but different food matrix, have opposite effects on health. This would contribute to explain the current controversial results on the health effects of dairy products.

3. Aims of this thesis

The primary goal of this thesis is to design a refined version of the MDS to more precisely assess the association of a Mediterranean-like diet with CVD risk in diverse North European populations. The score was refined by including, in its calculations, food items typical of a traditional Mediterranean diet (epidemiological contextualisation) and ingredients from mixed dishes and recipes (cultural adaptation) (see 4.2 in the Method section). This thesis describes the study of the association between the MDS and mortality in three different cohorts, such as the VIP (Tognon *et al.,* 2012), the MONICA-Copenhagen (Tognon *et al.,* 2014b) and the H70 cohorts (Tognon *et al.,* 2011). In addition to total mortality, the results of the association between the MDS and CVD mortality (Tognon *et al.,* 2012; Tognon *et al.,* 2014b) and incidence (Tognon *et al.,* 2012) are also described.

The second aim was to study the association between total mortality and intake of different types of dairy products. Responsive to recent results showing a direct association between milk intake and mortality, and the opposite for cheese intake, I aimed to confirm this result in the VIP (Tognon *et al.,* 2017) and H70 studies (Tognon *et al.,* 2018).

Finally, I aimed to determine whether the benefits of adherence to a Mediterranean diet can be evidenced across the life course. An appraisal of the utility of the MDS to determine associations with obesity in children (as a potential early risk factor of future CVD) in addition to the association between diet, longevity and chronic disease among adults and the elderly, confer a full life course perspective to this thesis.

4. Subjects and Methods

4.1 Population studies

This thesis assimilates research evidence from analyses conducted (and published) using data collected in five population studies (Ahrens *et al.,* 2011; Rothenberg *et al.,* 1997; Johansson *et al.,* 2002; Stegmayr *et al.,* 2003; Eriksson *et al.,* 2011) on more than 100,000 subjects of different ages from childhood to senescence (2 to 70 years) all of whom were of Caucasian ethnicity.

All aims (section 3) required use of dietary information collected in the above cohorts. Methods varied both in type of assessment method used, period of follow up and complexity of analysis therefore possible. Table 1 summarises data used in address of each aim alongside a link to references published from this work.

Table 1: Characteristics of the studies included in this thesis

The table lists the cohort studies used in this thesis together with the countries where participants have been recruited, their age range (years) at recruitment, and the number of participants in each cohort, both total and stratified by sex. The dietary assessment methods used in each cohort are also listed (FFQ = Food Frequency Questionnaire, 24HDR = 24h Dietary Recall). The table also clarifies the aims of this thesis in which each cohort was involved.

	Country of residence	Age range at recruitment (years)	Subjects with dietary data (n) (W = Women; M = Men)	Dietary assessment	Thesis aim	References
Västerbotten intervention study (VIP)	Sweden	25-74	100,256 (51,131 W; 49,125 M)	FFQ	1, 2	(Tognon *et al.,* 2012; Tognon *et al.,* 2017)
MONICA-Copenhagen	Denmark	30-60	1,849 (948 W; 901 M)	7-day Food Record	1	(Tognon *et al.,* 2014b)
Gerontological and Geriatric Studies in Gothenburg (H70)	Sweden	70	1,277 (714 W; 563 M)	Diet History	1, 2	(Tognon *et al.,* 2011; Tognon *et al.,* 2018)
Northern Sweden MONICA[2]	Sweden	25-74	12,263 (6,230 W; 6,033 M)	FFQ	2	(Tognon *et al.,* 2017)
Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS (IDEFICS)	8 EU countries	2-9	14,972 (FFQ) (7,351 W; 7,621 M) 7,940 (24HDR) (3,899 W; 4,041 M)	FFQ/24HDR interviews	3	(Tognon *et al.,* 2014a; Tognon *et al.,* 2014c)

[2] MONItoring trends and determinants in CArdiovascular disease.

The primary outcome for address of aim 1 was mortality and CVD and its association with adherence to a Mediterranean-like diet, for aim 2 it was total mortality and its association with dairy product intake and for aim 3 it was childhood overweight and obesity. Table 2 summarises the methods used in each study for these key outcomes as well as for other cofactors (e.g., smoking status, physical activity, BMR calculations) used in analyses presented (statistical methods are summarised in section 4.6).

Table 2: Summary of the methods used in each population cohort

The table lists all cohorts included in this thesis and summarizes how the primary research outcomes were defined (i.e., morbidity, mortality and childhood overweight including obesity), which anthropometric and lifestyle variables were collected as well as other relevant details (e.g., genetic analyses).

Study	Outcome	Anthropometry (measured)	Lifestyle variables (self-reported)	Other details
VIP	Mortality cases up to 31/12/07 (Swedish National Registry of Causes of Death). Total CVD: 390-438 (ICD9) or ICD-10 codes I00-I69. Max follow-up for total mortality: 18 yrs (17 yrs for CV mortality). 6,892 of 103,256 ppts (6.7%) died during follow-up period (Tognon *et al.,* 2012).	Weight (kg); height (m); BMI: normal weight (BMI < 25 kg/m^2), overweight (BMI ≥ 25 to <30 kg/m^2) and obese (BMI ≥ 30 kg/m^2). Consistent equipment brands and calibration methods used in all centres.	Smoking: Never, past, present Physical activity (Cambridge Index of Physical Activity): inactive, moderately inactive, moderately active and active subjects (InterAct Consortium *et al.,* 2012). PAL: 1.6 = cut-off for high physical activity. Baseline BP, lipid profile and OGT after an overnight fast (Norberg *et al.,* 2010).	Genotyping: N=7,404 (random sample). Education: Self-reported and dichotomised into university degree vs lower levels.
MONICA Copenhagen	Data from the National Patient Registry of Hospital Discharges, Cause of Death Register and Central Person Register. Cause-specific cases of death: 1. Total CVD: 390 – 458 (ICD8); I00 – I99 (ICD10); 2. MI: 410 (ICD8) and I21 (ICD10); 3. Stroke: 430 – 434 and 436 (ICD8) and I60 – I64 (ICD10). Average follow-up duration: 14 years Most recent update: 08/07/07. Survival time (years) parameterised as lifetime until death or censoring event, with	Body weight (kg), height (m). Sub-sample (n = 1,348 subjects): change in body weight at 11 years follow-up. BMR (kcal/day) Schofield equations for subjects aged 30–59 years (Schofield, 1985): Men: 11.5 x weight in kg + 873; Women: 8.3 x weight in kg + 846.	Smoking and leisure time activities: standardised questionnaire. PAL: inactive, light (light activity < 4 h/week), moderate (light activity > 4 h/week), and energetic activity (strenuous exercise > 4 h/week). Duplicate measures of BP. Total, HDL cholesterol and triglyceride after 12 hrs fast. Total: HDL-cholesterol > 4: indicative of a risky lipid profile.	Food intake levels (FIL): Ratio of reported energy intake to BMR. Education: Self-reported (number of years regular schooling) categorised as: 0-7, 8-11, and ≥ 12 years.

Study	Outcome	Anthropometry (measured)	Lifestyle variables (self-reported)	Other details
	left truncation[3] at the age of entry into the study being equal to baseline examination.			
H70	Mortality cases (n = 630) from the national death registration system. Average follow-up: 13.2 yrs Most recent update: May 21st, 2010	Body weight (kg) and height (m) BMR (kcal/day) standard equations for subjects aged 60–74 (FAO/WHO/UNU, 2001): Men: 0.0499 x weight in kg + 2.93; Women: 0.0386 x weight + 2.875.	Physical activity: active vs. inactive (Dey et al., 2001). Smoking: ever and never.	Education: self-reported; dichotomised into <6 schooling years and higher (≥6 years). Marital status: single, married, widow/widower, and divorced (self-reported).
IDEFICS	Cross-sectional analyses: Overweight including obesity based on Cole *et al.* (2000). Waist circumference and waist-to-hip ratio (WtHR) Percent fat mass Longitudinal analyses: Highest quintile of change at follow-up in BMI z-scores, waist circumference, WtHR, and percent fat mass.	Body weight (kg), waist circumference (cm), height (m), mean triceps and subscapular skinfold thickness (measured twice) (Stomfai et al., 2011). Percent fat mass estimated using the Slaughter equations (Michels et al., 2013): Boys: 0.783 x [triceps + subscapular] – 1.7 Girls: 0.546 x [triceps + subscapular] + 9.7.	Physical activity levels: sum # hours spent playing outdoors or in sports clubs (Burdette et al., 2004). BMR (kcal/day), Schofield equations (Schofield, 1985): Boys: 19.59 weight (kg) + 1.303 height (cm) + 414.9 Girls: 16.969 weight (kg) + 1.618 height (cm) + 371.2.	Parental education: Standardized across countries based on ISCED (UNESCO, 1997). Country-specific parental income levels: average net equivalence income, based on the median income and poverty line from each country (Ahrens et al., 2011). Parental migration status: self-reported.

Abbreviations: Body Mass Index (BMI), Cardiovascular disease (CVD), Basal Metabolic Rate (BMR), Waist-to-hip ratio (WtHR), High-density lipoproteins (HDL), International Standard Classification of Education (ISCED).

[3] Left-truncation at entrance age means that the individuals will be included in the risk set (i.e. at risk for disease outcome) only from a certain value of age on, which in this case was the age at recruitment. Left truncation was used in this cohort because some individuals had already experienced the CVD event at the time of study recruitment.

4.1.1 Västerbotten Intervention Program (VIP)

The VIP is an ongoing study that started in 1985. At the time when the project described in this thesis was started, 112,822 men and women living in the Västerbotten region (Sweden) had been recruited. Every year, all citizens turning 40, 50, 60, and 70 years who are registered with primary care centres or are registered as living in this region are invited to participate. In 2008, only 70-year-olds were invited to participate. Average recruitment rate was 59% of the invited subjects and a comparison with non-participants using census data revealed very little selection bias (Weinehall *et al.,* 1998). Data collected from health examinations included measurement of anthropometry, blood pressure, blood lipid profiles, plus an oral glucose tolerance test (OGTT). A standardised questionnaire about diet, lifestyle, and health conditions was filled out by each participant.

Detailed dietary assessment data was collected using an 84-food-item FFQ until 1997, when a 64-66 item questionnaire was developed and adopted. Of the 20 food items excluded in the shorter version, 12 were merged with another item. The latter included a few foods that fed into the MDS calculations as atypical Mediterranean foods, such as cheese spread, soft whey cheese, liver, kidney, blood-based foods plus one typical Mediterranean food item (i.e., shellfish). The FFQ was validated by comparison with data from ten repeated 24HDR, and by measuring plasma beta-carotene levels (Johansson *et al.,* 2002).

Food intake frequencies were reported on a 9-level scale, and portions were estimated using pictures of a plate containing increasing amounts of different types of food (Johansson *et al.,* 2002). Total energy intakes (kcal/day) were calculated by multiplying the reported frequencies of intake and the food composition data provided (Swedish Food Agency, 2020). Estimated intakes of energy, nutrients, vitamins, and minerals have been

validated against both repeated 24-h dietary records and biological markers (Johansson *et al.,* 2002, 2010; Wennberg *et al.,* 2009; Klingberg *et al.,* 2013).

4.1.2 MONICA studies

This cohort is part of the MONICA international project (Böthig, 1989)[4] and included 1,853 subjects aged 25-74 years (51.3% women) born in 1922, 1932, 1942, and 1952 and living in the Copenhagen County (Eriksson *et al.,* 2011). The overall recruitment rate was 83% (Heitmann, 1991).

In the MONICA-Copenhagen cohort all participants went through a health examination at recruitment. The latter included the drawing of a blood sample. A second health examination was performed after five years (Jensen and Jørgensen, 1991).

Seven-day food records reporting average household weights of 19 frequently-consumed foods were used to collect information on diet. Nine food groups were included: cereals and bread, vegetables, fruit, dairy products, meat (including poultry and fish), cold cuts, drinks, and miscellaneous food items. Instructed by a trained dietician, participants were invited to report any other food that was not mentioned in the food record. Weighed estimates were preferred, with guided estimation where this was not feasible, during a typical week including a minimum of non-habitual social activities. A trained dietician

[4] The MONICA (Multinational MONItoring of trends and determinants in CArdiovascular disease) project is an international study coordinated by the WHO which includes a cohort in the Västerbotten and Norrbotten regions. The scope of this large international study was to measure the trends in cardiovascular mortality and coronary heart disease across different countries, as well as to study the association with known risk factors, socioeconomic features and other factors.

manually checked and coded all food records. Validation was performed by comparison of 24-h urine nitrogen and calculated total protein intakes (Jørgensen *et al.,* 1992).

The Northern Sweden MONICA cohort included 12,263 subjects aged 25-64 years selected from population registries in Northern Sweden (Stegmayr *et al.,* 2003). Methods used were consistent with those described for the Copenhagen cohort with the exception of dietary assessment methods which were equivalent to those used in the VIP study.

4.1.2 Gerontological and Geriatric Population Studies in Gothenburg (H70)

The H70 study was started in the 70's with the goal of studying mental, physical and social health, as well as their determinants. It includes 4 birth cohorts (Rydberg Sterner *et al.,* 2019) of subjects aged 70 years and born in 1901, 1911, 1922, and 1930 living in Gothenburg (Sweden) invited to participate in a health examination conducted in, 1971, 1981, 1992 and 2000, respectively (Rothenberg *et al.,* 1997; Rothenberg *et al.,* 1998; Eiben *et al.,* 2004, 2005). Recruitment rates decreased from 84 - 86% in the earliest birth cohorts to 65% in the most recent one. This is a general phenomenon that has been observed in cohort studies conducted over the past decades thought to be related to the increasing number of surveys people are invited to, decreased mistrust in handling of personal information, and increasing time-pressures.

All subjects underwent a diet history examination, validated by heart rate monitoring, activity diary, and doubly-labelled water. This survey provided a thorough assessment of usual intakes for 224 food items and mixed dishes (Rothenberg *et al.,* 1997; Rothenberg *et al.,* 1998; Eiben *et al.,* 2004, 2005). Validation by heart rate monitoring assumed a linear relationship between heart rate and VO_2 and, consequently, with total energy expenditure

(Bradfield *et al.,* 1970). Individual regression lines between heart rate and VO_2 or total energy expenditure were obtained by measuring heart rate simultaneously with oxygen consumption or energy expenditure during treadmill walking. This method was validated, in turn, by indirect calorimetry (Elmståhl, 1987). The time spent doing each type of daily physical activity recorded in the activity diary by each participant was multiplied by the corresponding physical activity ratio (Tontisirin, 2001) then summed to estimate the total energy expenditure. Doubly-labelled water estimates were compared with energy intakes to identify over- and under-reporters (Goldberg *et al.,* 1991). All subjects reported their dietary intakes in a multi-pass interview process which helped ensure a high level of completeness.

4.1.3 Identification and prevention of Dietary- and lifestyle-induced health EFfects In Children and infantS (IDEFICS)

This multi-centre intervention study recruited 16,220 children ages 2-9 years in eight EU countries: Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium, and Estonia. The recruitment rate was 51% (Ahrens *et al.*, 2011). IDEFICS was initiated to investigate the risks and long-term consequences of overweight and obesity in children. A baseline survey assessing the children's diet, lifestyle and health by specific questionnaires was completed in 2008. Since the survey was repeated after two years in 9,114 children, differences in anthropometry between baseline and follow-up were calculated.

The IDEFICS protocol included a 2-year intervention phase that was run between the initial recruitment and the first follow-up. In this phase, in which one intervention area per country was matched to one control. The intervention was designed as a non-randomized trial and delivered in school and community settings, aiming to achieve a reduction in the

prevalence of overweight and obesity. Data from both intervention and control communities are included here.

24HDR questionnaires were self-administered by parents using computer-based "Self-Administered Children and Infant Nutrition Assessment" (SACINA) software validated by comparing the agreement between energy intakes (EI) from the 24HDRs and total energy expenditures (TEE) estimated through doubly-labelled water (Börnhorst *et al.,* 2014; Westerterp, 2017). The EI:TEE ratio was used to identify adequate, low- and high-energy reporters (Sjöberg *et al.*, 2003). Overall, 78% of children were classified as adequate energy reporters (ratio = 1, neutral energy balance), whereas 14% reported energy intakes higher than their total energy expenditure, and only 8% reported EI lower than TEE.

Pictures (country-specific where necessary) were used to facilitate the choice of portion sizes. Food group, nutrient and energy intakes during the previous 24 hours were quantified using country-specific food composition tables, with the exception of Hungary where the German food composition tables were used (Hebestreit *et al.,* 2011) because common European food tables do not exist. Consumption of meals in school and pre-school was investigated by means of direct observations except in Cyprus where school ended before lunch. Trained survey personnel interviewed teachers and school kitchen staff to collect data about what children ate at school. Each day, teachers were given a list of the children whose parents were going to be interviewed the day after so that they could keep a diary of what the selected children consumed during school time. Recalls characterised by implausible intakes (for example, 485% energy from fat) or by under-reporting or over-reporting (n = 1,816) based on Goldberg cut-offs adapted to children (Börnhorst *et al.*, 2014) were excluded. 14,863 24HDR interviews (of which 11,669 were first 24HDR) were linked to food composition tables specific for each country to estimate nutritional intake.

A self-administered dietary questionnaire (Children's Eating Habits Questionnaire FFQ, CEHQ-FFQ) was filled out by the parents of 14,972 children (7,351 girls) when the latter underwent a physical examination. This was designed to investigate consumption frequencies of 43 foods under parental control together with other diet-related habits (e.g. eating in front of the TV). The questionnaire was tested only for relative validity (by comparison with 24HDR) and reproducibility (in a sub-sample of parents of 258 children) before the baseline examination, showing both valid and reproducible estimates (Lanfer *et al.,* 2011; Bel-Serrat *et al.,* 2014). For each of the 14 groups that reported in the CEHQ-FFQ and each recall day, intake quartiles were calculated and cross-tabulated (CEHQ-FFQ vs 24HDR intakes) to identify the level of agreement between the two dietary assessment methods. Both crude and de-attenuated correlation coefficients and weighed kappa statistics (kw) were also calculated. On average the intake estimates from the CEHQ-FFQ were higher than the 24-HDR for most food groups. Among children aged 2 to <6 years, de-attenuated Pearson correlation coefficients ranged from 0.01 for sweetened fruit to 0.48 for sweetened milk (mean = 0.25) and from 0.01 (milled cereal) to 0.44 (water) in children aged 6–9 years (mean = 0.23). Approximately 31-32% of food group intakes were assigned to the same quartile in both age groups (i.e., 2- <6 and 6-9 years) and classification into extreme opposite quartiles was ≤12% for all food groups in both age groups. Mean kw was 0.20 and 0.17 for 2 – <6 and 6–9-year-olds respectively. The results of the validation study suggested a reasonable level of agreement between the CEHQ-FFQ and the 24HDR, though this counts as a relative validity. Methods were validated by objective methods. The 24HDR was validated by doubly-labelled water, which found good levels of agreement between total energy expenditure and energy intakes, with larger differences in overweight and obese children (although the maximum difference found was only 86 kcal, Börnhorst *et al.,*

2014). Milk consumption frequencies estimated by the CEHQ-FFQ were predictive of urinary calcium to urinary creatinine ratio and urinary potassium to urinary creatinine ratios, in a multivariate regression analysis adjusted for age, gender, study centre, soft drink consumption and frequency of main meals consumed at home (Huybrechts *et al.,* 2011).

4.2 Design and refinement of the MDS

Adherence to a Mediterranean-like diet in epidemiological studies has been performed using several adaptations of the original MDS (Trichopoulou *et al.,* 1995) and used in a plethora of studies (Dinu *et al.,* 2018). The MDS is calculated by assigning an increasing number of points when intakes of legumes, total grains, fruit, and vegetables exceed population-specific medians. Additional points are assigned to subjects who exceeded the population-specific median for MUFA:SFA ratio and to those who reported intakes of dairy products and meat products below the respective population-specific medians. More recent modifications include an additional point for high fish intakes (Trichopoulou *et al.,* 2003).

A refined version of the MDS was developed to enable comparisons of the Mediterranean diet with cultural adaptations typical in Nordic population studies. The most extensive score adaptations were done in the MONICA-Copenhagen and H70 studies. In both, detailed dietary assessment methods (i.e., 7-day food records and diet histories, respectively) enabled this. The H70 diet history included questions about food beliefs, attitudes, appetites, kitchen facilities followed by a detailed interview on portion sizes and consumption of 224 different food items (Rothenberg *et al.,* 1993). In both the H70 and MONICA-Copenhagen studies plus IDEFICS, intakes of mixed dishes and recipes were also captured. Commonly, these dishes included ingredients considered typical (e.g.,

vegetables) and atypical (e.g., meat) Mediterranean foods. Individual ingredients were therefore scored individually during the MDS calculations. An example of this is the coding of "Pasta Bolognese" as meat (the sauce contributing to total meat intake), total cereal (pasta) and vegetables (tomato sauce). To enable a more detailed evaluation of what each fat type comprised with regard contributing foods as suggested by Knoops *et al.* (2004), I also incorporated total unsaturated fatty acids including polyunsaturated fatty acids (PUFA) alone and with monounsaturated (MUFA + PUFA). This allowed a detailed assessment of adherence to the principles of the Mediterranean diet, which is rich in both olive oil and fish. Since grains are usually consumed unrefined in a traditional Mediterranean diet, wholegrain cereal intakes (rather than 'total cereal intakes') were used when no information on cereal type was available. Finally, the contribution of wine instead of total alcohol intake was established to avoid confounding by health effects of other types of alcoholic drinks (e.g., beer or spirits).

In the IDEFICS study, the MDS was calculated on data from either 24HDR or FFQ. For the former, a further adaptation was required since only food frequencies were available from the FFQs. Therefore, a second version using daily food frequencies instead of quantities (fMDS) was created for use in analyses based on FFQ data. Energy intakes could not be obtained from the FFQs; therefore, for the analyses with the latter I adjusted intake frequencies by dividing them for the total intake frequencies of all foods included in the questionnaire. Following the method used in the adult survey, 1 point was given to subjects whose adjusted intake frequencies were greater than (or smaller than for meat and dairy products) the age- and sex-specific medians calculated on the whole cohort. The final score was the sum of these points and called fMDS (i.e., frequency-based MDS).

Due to the characteristics of the CEHQ-FFQ used in the IDEFICS study, none of the MDS refinements performed in the score used in adults were possible when working with FFQ data. The data from 24HDR allowed, instead, two score refinements i.e., the inclusion of ingredients from mixed dishes and of PUFA intakes in the score calculations.

Not all the score refinements described above were possible in all the population studies included in this thesis. Table 3 provides a summary of the score refinements (e.g. use of ingredients from mixed dishes and recipes) in each cohort.

Table 3: Summary of the refinements done to calculate the MDS in each study cohort

The table below clarifies which, among the refinements described in the introduction, were applied (green tick) or not applied (red cross) when calculating the MDS in each cohort included in this thesis. The original score did not include ingredients from mixed dishes in its calculations. In addition, the original score is calculated based on total alcohol and total grain intakes, and the fat ratio is calculated by dividing MUFA intakes with SFA intakes.

MDS adaptation	Population study and dietary assessment method				
	VIP (FFQ)	MONICA Copenhagen (Food records)	H70 (Diet history)	IDEFICS (FFQ)	IDEFICS (24HDR)
Ingredients from mixed dishes and recipes	X	✓	✓	X	✓
Wine instead of alcohol intakes	✓	✓	✓	-	-
Wholegrain instead of total grain intakes	✓	X	✓	X	X
PUFA in addition to MUFA intakes	✓	✓	✓	N.A.	✓

N.A. = Not Available.

4.3 Assessment of dairy product consumption

Analyses on dairy product intakes and mortality were performed in three cohorts: the VIP, the Northern Sweden MONICA, and the H70 studies. In the analyses on the VIP and the Northern Sweden MONICA studies, individual intakes of non-fermented milk, fermented milk (i.e., sour milk and yoghurt), cheese and butter were used in the analyses. Although these studies included subjects who were recruited over the course of several years, all questions about dairy product intakes have remained unchanged except high- and low-fat hard cheese intakes which were questioned separately from 1991.

In the H70 study, milk, sour milk, and yoghurt were combined into one variable (i.e., milk products) because intakes had not been assessed separately in all four birth cohorts. Total cheese intakes were calculated by summing intakes of all cheese types. Fat intakes from both milk products and cheese were also calculated.

4.4. Dairy product intake and mortality

Data from three adult studies were used: VIP (Norberg *et al.*, 2010), Northern Sweden MONICA (Stegmayr *et al.,* 2003) and the H70 study (Rothenberg *et al.*, 1997). Data from the first two cohorts were merged because the two studies recruited subjects in the same geographical area and performed identical health examinations and surveys (Tognon *et al.*, 2017b).

VIP and Northern Sweden MONICA study –In the analyses on dairy product subtypes, total mortality up to the 31st December 2014 was used. The final dataset included 6,892 cases (41.7% women) with a mean follow-up time of 13.7 ± 6.8 years. For the analyses on dairy products, the Healthy Diet Score was calculated to assess healthy eating habits, based on the method described by (Nettleton *et al.*, 2013). Study-specific (i.e., VIP or MONICA)

quartile rankings for intakes of foods with favourable health effects (i.e., wholegrain cereals, fish, fruit, vegetables, and nuts/seeds) as well as foods with unfavourable health effects (i.e., red/processed meats, sweets, sugared beverages, and fried potatoes) were calculated. Intake quartiles of favourable food items were assigned ascending points (0, 1, 2, 3), whereas the opposite was done for unfavourable foods. The sum resulted from the sum of these points and ranged between 0 and 27.

Gerontological and Geriatric Population Studies in Gothenburg (H70) – For the analyses on dairy product intakes and mortality, total mortality was updated to 2010 with a total of 833 deaths recorded.

4.5 MDS and overweight during early life

Inclusion of the IDEFICS cohort provided an opportunity to include a life course perspective to this thesis. At the time when this thesis is being written, IDEFICS is still one of the largest epidemiological studies performed on children. In cross-sectional analyses, the following dichotomous outcomes were used: (1) overweight and obesity, based on a BMI cut-off (defined as "iso-BMI") which corresponds to a value of 25 kg/m^2 at the age of 18 (Cole *et al.*, 2000) and (2) a Waist-to-Height Ratio (WtHR) higher than normal (> 0.5) (Yan *et al.*, 2007). Waist circumference and percent fat mass were used as continuous outcomes.

In prospective analyses, dichotomous outcomes were defined as the highest quintile of change during follow-up in BMI z-scores, sex- and age-adjusted BMI, waist circumference, WtHR and percent fat mass from skinfold measurements. BMI change was adjusted for sex and age by subtracting the change in sex-specific and age-specific BMI medians from

the change in BMI for the same period. Age was dichotomised based on the following arbitrary cut-offs: 2-5 years (pre-school age) vs. 6-9 years (school age).

4.6 Statistical analyses

All statistical analyses described in this thesis have been performed using version 9.0 of the SAS statistical software (SAS Institute, Cary, NC, USA).

4.6.1 AIM-1: Refinement of the MDS

MDS, all-cause, and CVD mortality in the VIP

Cox proportional hazard models were used to compare the association between the original and refined versions of the MDS and mortality. Cox models were adjusted for age, smoking status, obesity, physical activity and education. Both total and CVD mortality were studied (all types, MI, stroke).

Participants were subsequently divided into five groups, depending on the number of reported healthy lifestyle-related factors (between 0 and 4), including: never being a smoker, BMI < 30 kg/m^2, PAL ≥ 1.6 and MDS > 4. The group with no healthy factors was used as a reference so that HRs calculated for the other groups represent a comparison with the reference. All the models mentioned so far included dichotomous dummy variables for missing values in the smoking status and physical activity variables coded as 0 (non-missing value) and 1 (missing value). This allowed me to retain the maximum possible number of participants.

Many sensitivity analyses were run, the first one being the adjustment for variables describing conditions likely related to a change in the diet, such as high baseline

cholesterol, diabetes (or glucose impairment), hypertension, or pharmacological treatment for heart disease. Next, I excluded all subjects who died during the first two years of follow-up, to avoid confounding due to the presence of subclinical diseases at baseline. In the third sensitivity analysis, the main analyses were repeated including only "adequate" reporters, which were identified based on three progressively more stringent definitions: the lowest 30%, 50% or 65% FIL-to-PAL ratio.

MDS, total mortality and CVD in the MONICA-Copenhagen study

Cox proportional hazard models were used to compare the association between two versions of the MDS (i.e., original and refined) and the following outcomes: all-cause mortality, as well as incidence and mortality for CVD (all types, MI, and stroke). Cox models were adjusted for sex, age, education (lowest education levels vs higher levels), smoking status (never smoked, < 30 pack/years, or ≥ 30 pack/years), physical activity (inactive, light, moderate, energetic) and obesity (BMI > 30 kg/m^2). A risk score was calculated by giving one point for each of the following characteristics: ever been a smoker, PAL < 4 h/week, BMI ≥ 30 kg/m^2, and MDS ≤ 4. The score was tested in sex-adjusted Cox proportional hazard models to assess the cumulative effect of unhealthy lifestyle factors on the risk of all-cause mortality. Sensitivity analyses included the exclusion of subjects who died during the first two years of follow-up, and confirmation of results in a sub-sample of "adequate reporters" based on three increasingly more stringent definitions: the lowest 30%, 50%, and 65% of the FIL: PAL ratio.

MDS and mortality in the H70 study

The association between two versions of the MDS (i.e., original and refined) and total mortality was investigated in Cox proportional hazard models. The latter were adjusted for sex, birth cohort, baseline BMI, and waist circumference, smoking status, physical activity level, education, and marital status. To exclude any influence on mortality by sub-clinical conditions at baseline, the main model was re-run after excluding all subjects who died during the first two years of follow-up. The analyses were also repeated by censoring at 8.5 years (i.e., the longest follow-up time in the latest cohort) and not at a fixed date. The latter analysis was meant to exclude the possibility that the different follow-up durations in each birth cohort could have influenced the results. Further sensitivity analyses included adjustment for the 'activities of daily living' variable[5] and potential mediators of CVD: such as baseline blood pressure, fasting glucose and lipid levels (total cholesterol and triglycerides), and body weight and waist circumference change at follow-up.

4.6.2 AIM-2: Dairy product intake and mortality

Analyses conducted in the VIP and Northern Sweden MONICA studies dataset

Reported intakes of non-fermented milk (i.e., the regular milk sold in all supermarkets), fermented milk (yoghurt and sour milk), cheese and butter were categorised based on the following arbitrary thresholds: (1) less than once a week, (2) between once a week and once a day, (3) between once a day and 2.5 times a day, and (4) more than 2.5 times a day.

[5] The Katz index was used to assess the activities of daily living variable. This score is based on the need of help for at least one out of nine different activities, such as shopping, house cleaning, transport, washing, using the toilet, cooking, dressing, feeding, and ambulation (Sonn and Asberg, 1991).

No distinction between fermented and non-fermented cheese intakes was made because this information was not available.

For each dairy product and intake level (16 groups overall), we calculated: the number of participants, the proportion of men and women, the mean and standard deviation of age and BMI, as well as the prevalence (%) of smokers, highly-educated, and physically inactive subjects. For each dairy product and intake level, means and standard deviations of the following variables: % energy from total, saturated, and trans fatty acids; wholegrain, vitamins C and D, lactose intakes per 9 MJ, and healthy diet score were also calculated. Intakes were standardised for sex, age, and BMI. Three groups of subjects were identified as exclusive drinkers of high (3%), medium (1.5%), and low fat (0.5%) milk and the following variables were summarised for each group: number of observations, number of deaths, age, BMI, smoking status, education, physical activity level, marital status, Swedish origin, % working days spent on sick leave, milk intake, healthy diet score, intakes of selected nutrients (vitamin C and D, lactose, fat), and wholegrain cereal intake. Intakes were standardised for sex, age, and BMI. Significance levels (p-values) for linear trends were calculated.

Associations between intakes of non-fermented milk, fermented milk (i.e., soured milk and yoghurt), cheese as well as butter and all-cause mortality were tested using Cox proportional hazard models. The latter were run after excluding individuals with missing data for smoking status (1.1%), education (0.7%), and BMI (0.5%) on a total of 101,019 subjects (6892 deaths). The potential confounders included in the model were sex, age at baseline, BMI (categorised into normal weight, overweight, obese), year of recruitment, smoking status (current, ex, never smokers), education (university degree vs lower education level), and total energy intake (KJ/day). Dummy variables for subjects with

missing values for smoking status and physical activity were created for the use in sensitivity analyses where we tested the effect on HRs when these subjects were retained into the model.

The analyses were repeated in a sub-sample including 41,676 subjects who reported to exclusively consume one type of non-fermented milk, such as high-, medium-, or low-fat milk only. The few subjects reporting intake frequencies lower than once a week and exclusive for one type of non-fermented milk were excluded from these analyses. By comparing HRs across the four categories of intake previously described[6], we tested whether any dose-response effect was present.

Several sensitivity analyses were performed, such as the exclusion of subjects with missing data for physical activity (11%), younger than 35 years of age, who reported extreme intake frequencies (i.e., > 99th percentile), of non-Swedish origin, who died during the first two years of follow-up, and who were examined before 1991. At this time, an additional question was asked for cheese intake (i.e., high- and low-fat hard cheese) and a transition from high- to medium-fat milk took place in the Swedish society. Other sensitivity analyses included adjustment for the healthy diet score (Nettleton *et al.,* 2013) or intakes of vitamin D, calcium or lactose (the latter as a proxy for galactose). To test potential residual bias from lifestyle-related factors, we repeated the analyses in a homogeneous sub-sample of non-smokers, with more than nine years education (equivalent to the compulsory school in Sweden), and a healthy diet score above the median (i.e., > 12).

[7] Or baseline waist circumference in the analyses on waist circumference change, etc.

Analyses conducted in the H70 study

Associations between cheese and milk products (i.e., combined intakes of non-fermented milk, soured milk, and yoghurt) and all-cause mortality were tested, as continuous variables, in Cox proportional hazard models. These analyses were adjusted for sex, birth cohort, BMI, smoking status (never vs ever smokers), marital status (married vs not married), physical activity level (low vs higher activity), and education (basic vs higher education). Further adjustments for adequate protein intakes (> 1.2 g/kg body weight), alcohol and fat intakes (as continuous variables) were done in separate models.

Both cheese and milk intakes were categorized to make HRs more easily interpretable in terms of portions required to increase or decrease the risk. Cheese intakes were divided by 10 g, an amount corresponding to what we estimated being an average single portion in Sweden, where cheese is usually cut in thin slices. In contrast, milk product intakes were divided by 100, corresponding to approximately 1/5 of a pint as done in previous studies (Guo *et al.,* 2017).

All Cox models were run using the STRATA command in the PROC PHREG procedure (version 9.4, SAS statistical software) to allow baseline risks for each birth cohort to vary without reducing the power of the analyses. This command considers potential differences in the risk of all-cause mortality among different birth periods. The results were stratified by follow-up duration, due to unfulfillment of the assumption of proportional hazards required by the Cox model. The association between intakes of fat from either cheese or milk products and mortality was also tested in Cox models adjusted for the covariates mentioned above. Dummy variables were used to retain subjects with missing information for potential confounders, although the analyses were also repeated using multiple imputation (Stata, 2013) to estimate missing values. Effect modification by the participants'

nutritional status was also assessed by testing the interaction between both BMI and high protein intake (> 1.2 g/kg/day) (Bauer *et al.,* 2013) with cheese and milk product intakes. The above models were also repeated after excluding subjects who died during the first two years of follow-up.

Dose-response was tested in Cox models adjusted for the above covariates and comparing sex-specific tertiles of cheese and milk product intakes (first tertile as reference). Non-linearity was also investigated comparing likelihoods of three Cox models: (1) including cheese and milk product intakes as continuous variables, (2) including intakes (as quadratic terms), (3) using spline regression models (Marsh and Cormier, 2001) to estimate HRs of four intervals of intakes. The latter three models were compared by a likelihood ratio test, assuming the model with the highest likelihood was the one producing the most accurate prediction.

4.6.3 AIM-3: MDS and overweight during early life

Cross-sectional and longitudinal association of fMDS with indicators of obesity in the IDEFICS study

An ANOVA adjusted for sex, age, parental income, and education was used to test the differences between fMDS among study centres. The centre which showed the highest fMDS (i.e., Sweden) was compared to all the others using the Dunnett post-hoc test. In a logistic regression analysis, adjusted for the same covariates used in the ANOVA plus a variable describing the study centre, I tested the association between high (> 3) fMDS levels and the highest quintiles of changes in BMI z-scores, waist circumference, percent fat mass,

and WtHR. The estimates of the association between overweight including obesity and high fMDS (both combined and centre-specific) were depicted in a forest plot.

Using logistic regressions, I tested the association between baseline fMDS with the highest sex- and age-specific quintiles of anthropometry change during follow-up. The anthropometrical variables tested in these analyses included: BMI, BMI z-scores, waist circumference, WtHR and % fat mass. The covariates included in this model were sex, baseline age, parental education and income, study centre, participation in the intervention study, as well as sex- and age-specific BMI z-scores[7].

Since no information about intakes of fatty acid subtypes was available, it was not possible to calculate a ratio between saturated and unsaturated fats. However, this information was available in the 24h recall dataset performed in a sub-sample of 9,082 children. In this sub-sample, I calculated the ratio between polyunsaturated (MUFA + PUFA) to SFA. I subsequently stratified the dataset in three categories based on the fMDS and including a similar number of children: from 0 to 2, 3, or between 4 and 6. By linear regression, adjusted for sex, age, study centre as well as parental income and education, p for trends across fMDS categories were calculated.

Since the CEHQ-FFQ did not enquire about the food consumed at school, additional sensitivity analyses were run adjusting for the number of meals consumed at home. Three further sensitivity analyses were performed, the first in a sub-sample excluding the highest 5% of BMI, waist circumference, WtHR or percent fat mass (depending on the outcome tested). The second adjusting for having at least one immigrant parent, a condition potentially associated with different food culture, and lower socioeconomic status. The

[7] Or baseline waist circumference in the analyses on waist circumference change, etc.

third analysis was performed adjusting for a variable which summarised the average number of hours each child spent in sports clubs or playing outdoors. The latter was considered a proxy for physical activity. Values > 7 h/day were considered implausible, and the subjects reporting them were excluded.

Adherence to the Mediterranean diet in the IDEFICS study based on 24HDRs

Descriptive analyses included the calculation of the prevalence of MDS > 3[8] during the first 24h dietary recall stratified by sex, age, study centre, and parental migration status. Only 18.5% of all recalls were collected during either Saturday or Sunday when the diet is expected to differ compared to the rest of the week. Therefore, the prevalence of high adherence during these days was not considered. Another descriptive analysis calculated the proportion of boys, pre-school age and children having highly educated and high-income parents, as well as children having at least one migrant parent with either high (> 3) or low (≤ 3) MDS. Prevalence of high adherence from the second 24h dietary recall and during weekdays was also calculated, stratified by sex and age. The analyses were repeated using a score calculated excluding potatoes.

4.7 Ethics

With the exception of the two earliest birth cohorts in the H70 study, where data was collected in 1971 and 1981, and therefore before the Declaration of Helsinki of 1989, all studies have been approved by local ethical committees. All participants whose data were

[8] An arbitrary threshold for high adherence to a Mediterranean-like diet.

included in this thesis gave their consent at recruitment. During the IDEFICS study, consent was collected from both parents and children, and both could refuse to participate in any specific protocol component (e.g. genetic tests).

5. Results

The results below are organized based on the aims described in section 3 i.e., refinement of the MDS, dairy product intake and mortality, and MDS and overweight in early life.

Table 4 cross references all results described in each paragraph of this section with the results published in each manuscript:

Table 4: Cross references to published results

In this table, the first column lists all paragraphs contained in the result section of this thesis. For each section, a cross reference to published results is also shown to guide readers retrieve the published source of the results presented. Cross-references to published results are reported as table number, figure number, or title of the specific paragraph in the result section of each published manuscript to which a reference is also given.

Paragraph	Cross references to published results
5.1.1	Table 3 in Tognon *et al.*, 2011
	"Descriptive analyses" in Tognon et al., 2012 and Tognon *et al.*, 2014b
5.1.2	Table 2 in Tognon *et al.*, 2012 and Tognon *et al.*, 2014b
	Table 5 in Tognon *et al.*, 2011
5.1.3	Table 2 in Tognon *et al.*, 2014b
	Table 3 in Tognon *et al.*, 2012
5.1.4	Figure 1 in Tognon *et al.*, 2012 and Tognon *et al.*, 2014b
5.1.5	"Supplementary analyses" in Tognon *et al.*, 2012
	"Sensitivity analyses" in Tognon *et al.*, 2014b
5.2.1	Table 2 in Tognon *et al.*, 2017
5.2.2	Tables 3 and 4 in Tognon *et al.*, 2017
5.2.3	Table 2 in Tognon *et al.*, 2018
5.2.4	Table 3 in Tognon *et al.*, 2018
5.2.5	"Sensitivity analyses" in Tognon *et al.*, 2017
5.3.1	Table 1 and Figure 1 in Tognon *et al.*, 2014a
5.3.2	Table 2 in Tognon *et al.*, 2014a
5.3.3	Table 3 in Tognon *et al.*, 2014a
5.3.4	Figure 2 in Tognon *et al.*, 2014a
5.3.5	"Additional analyses" and Tables 1S and 2S in Tognon *et al.*, 2014a
5.3.6	Table 1 in Tognon *et al.*, 2014a
	Table 2 in Tognon *et al.*, 2014c
5.3.7	Table 1 in Tognon *et al.*, 2014c

5.1 Aim 1: Refinement of the MDS

This section presents the results obtained from analyses conducted in three cohorts (VIP, MONICA-Copenhagen, and H70 studies), on the association between the MDS and total mortality (all cohorts), CVD mortality (VIP and MONICA-Copenhagen), and CVD incidence (MONICA-Copenhagen). These results were obtained comparing the refined and original versions of the MDS. My hypothesis was that use of a refined MDS would evidence stronger associations with both total mortality and CVD incidence and mortality compared to the original MDS used in other studies. The score refinement was based on including ingredients from mixed dishes and recipes, updating alcohol to consider wine intake, wholegrain instead of total cereals and including PUFA in the score calculations.

5.1.1 Cohort general characteristics

Of the three cohort studies used in this part of my study, two included participants recruited in Sweden (the VIP and the H70 cohorts) and one in Denmark (the MONICA-Copenhagen cohort). All three included approximately half men and women (women represented 51.4% of the VIP cohort, 51.3% of the MONICA-Copenhagen cohort, and 47.9% in the H70 cohort). The percent of obese subjects was 15.8% in the Swedish H70 study (which included only 70-year-old subjects), 8.2% in the VIP and 7.5% the Danish MONICA-Copenhagen study (both studies that included adult subjects). In comparison, the national obesity rate in 2018 was 14% in Sweden and 17% in Denmark (OECD/EU, 2020). Approximately one quarter of participants in the MONICA-Copenhagen study were never smokers (25.7%). In the H70 studies the proportions of never smokers were substantially higher among women compared to men with large variations across older and younger birth cohorts (17% to 31% of never smokers among men and 82% to 55% among women).

Finally, 29.6% of participants in the VIP reported having completed a university education (bachelor's degree or higher), whereas 30.5% of subjects included in the H70 study reported a level of education above the mandatory level.

5.1.2 Inverse association between the MDS and all-cause mortality

In total, 28 variants of the MDS have been used in observational studies, none of which have been refined to more accurately describe the context of a Mediterranean diet or have been culturally-adapted to the specific study population (Tognon *et al.,* 2011). Score variants (Zaragoza-Martí *et al.,* 2018; Jacobs, 2012) differ based on how typical and atypical Mediterranean foods have been grouped (e.g., potatoes together with vegetables, with grains or simply excluded) as well as based on the intake cut-offs used for the calculations of the MDS (e.g., medians or tertiles). Despite the large variability in the assessment of adherence to the Mediterranean diet, the latter has repeatedly been shown to be directly associated with a series of health-related outcomes in observational studies (Sofi *et al.,* 2008, 2010, 2014; Dinu *et al.,* 2018).

After refining the MDS to more accurately describe the context of a genuine, traditional Mediterranean diet and by adapting it to the culture of North European study populations (see section 4.2), I showed that the MDS was not associated with the risk of mortality in three Scandinavian studies, whereas a refined version of this score was inversely associated with this outcome. These results (see Table 5) were obtained, with similar HRs, in the VIP (refined score: HR = 0.95, 95% CIs: 0.93; 0.98) (Tognon *et al.,* 2012), MONICA-Copenhagen (refined score: HR = 0.93, 95% CIs: 0.88; 0.99; original score: 0.95, 95% CIs: 0.91; 1.00) (Tognon *et al.,* 2014b), and H70 studies (refined score: HR = 0.93, 95% CIs: 0.89; 0.98; original score: HR = 0.97, 95% CIs: 0.92; 1.02) (Tognon *et al.,* 2011). Refinement was feasible

to varying extents in these studies (e.g., intakes of mixed dishes were not available in the VIP cohort, and wholegrain cereal intakes were not available in the MONICA cohort). I used Cox models to compare the association of two versions of the MDS (refined and original) with outcomes including all-cause mortality, CVD incidence, and mortality. Models were adjusted for sex, age (or birth cohort in the H70 study), BMI, physical activity level, smoking status, and education. Cox proportional hazard models represent robust statistical approaches used by epidemiologists to assess the association between risk or preventive factors with either mortality (both total and cause-specific) or disease incidence (Cox, 1972) Previous observational studies had not been performed in Scandinavian study population, with the exception of Sjögren *et al.* (2010) who found an inverse association with total mortality. However, the external validity of this study was limited by the fact that this study was performed in a population of 70-year-old men only.

Table 5: Association between the MDS (refined and original version) and all-cause mortality

Cox proportional hazard models were to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between a one unit increase in both the refined and original version of the MDS and all-cause mortality. All analyses were adjusted for sex, age (or birth cohort in the H70 study), BMI, physical activity level, smoking status, and education. The MDS is included as a continuous variable. Both the total number of subjects and the number of mortality cases are reported.

Study	Score version	n subjects	n cases	HR[1] (95% CIs)
MONICA-Copenhagen study (Tognon *et al.,* 2014b)	Original	1,849	553	0.95 (0.91; 1.00)
	Refined	1,849	553	0.93 (0.88; 0.99)*
VIP (Tognon *et al.,* 2012)	Refined	77,151	2,376	0.95 (0.93; 0.98)***
H70 Gerontological Study (Tognon *et al.,* 2011)	Original	1,037	718	0.97 (0.92; 1.02)
	Refined			0.93 (0.89; 0.98)*

[1]All models were adjusted for a number of potential confounders such as sex, age (or birth cohort in the H70 study), BMI, physical activity level, education and smoking status. In the H70 study, the analyses were further adjusted for marital status. *$p < 0.05$. ***$p < 0.001$.

Key messages

- Several MDS version exist, but this is the first time they have been refined to accurately describe the context of the Mediterranean diet and culturally-adapted to the specific study population.
- Many studies have shown that the MDS is inversely associated with the risk of mortality and chronic disease. This is the first to be performed in Scandinavia.
- Refinement of the MDS in these studies was required to highlight any inverse associations with total mortality in the studies included in this thesis.

5.1.3 Inverse association between the MDS and CVD

Since adherence to a Mediterranean-like diet, assessed through a refined version of the MDS, was inversely associated with all-cause mortality, the next step was to understand whether these results could be confirmed for CVD incidence and mortality, which represents the leading cause of death that, in 2013, killed 17 million people globally (Roth *et al.,* 2015).

The refined score was calculated using intakes of wholegrain cereals and not total cereals, since unrefined grain consumption has been directly associated with CVD risk reduction (Temple, 2018). Similarly, high consumption of foods whose intakes are positively scored in the refined calculations, such as fish, nuts, legumes, fruit, and vegetables (as well as low intakes of red meat) are also inversely associated with the risk of CVD (Mozaffarian, 2016). Wine intakes, used instead of total alcohol intakes, typically reflect a responsible consumption pattern of alcoholic drinks in Mediterranean diets where wine is consumed

at meals and in moderate amounts. This may explain its inverse association with CVD risk (Augustin *et al.,* 2004; Hernandez-Hernandez *et al.,* 2015).

CVDs are a heterogeneous group of diseases; therefore, it was important to study risk and preventive factors separately for each subtype. In this thesis I chose to investigate myocardial infarction and stroke separately. According to a Cochrane meta-analysis, dietary interventions alone could produce a 13% reduction in stroke risk (Rees *et al.,* 2013) and Mediterranean-like diets highlight an inverse relationship with stroke risk (Kontogianni and Panagiotakos, 2014). A recent population study confirmed that a version of the MDS modified using similar principles to those illustrated here[9] inversely predicted the risk of myocardial infarction, heart failure, and ischemic stroke (Tektonidis *et al.,* 2015).

In these analyses, I used two cohorts: the VIP and the MONICA-Copenhagen studies (Tognon *et al.,* 2012; Tognon *et al.,* 2014b). In the MONICA-Copenhagen study, 553 deaths were identified: 223 of which were due to CVD (64 deaths for MI and 40 for stroke), while 755 incident CVD cases were detected during a follow-up period of 14 years on average (162 MI and 167 cases of stroke). In these analyses, the refined MDS was inversely associated with total CVD morbidity (HR = 0.92; 95% CIs: 0.87; 0.97) and mortality (HR = 0.87; 95% CIs: 0.79; 0.95), as well as with MI morbidity (HR = 0.89; 95% CIs: 0.80; 1.00) and mortality (HR = 0.79; 95% CIs: 0.66; 0.94). No association was found with stroke morbidity and mortality (Table 4). Contrary to what was found in the MONICA-Copenhagen study, no statistically significant association was identified in the VIP between the refined MDS and CVD mortality in men and women combined (Table 6). When analyses were stratified by sex however, statistically significant associations were found in women between the MDS

[9] i.e., based on high intakes of whole grain cereals, fruit, vegetables, nuts, fermented dairy products, moderate alcohol intakes and low red meat intakes.

and both total CVD (HR = 0.90, 95% CIs: 0.82; 0.99, 181 deaths) and MI (HR = 0.84, 95% CIs: 0.71; 0.99, 61 deaths). The MDS was not associated with stroke mortality in either men or women.

Despite previous evidence from observational studies showing that diet is a risk factor for stroke (Hankey, 2017), my analyses did not show any statistically significant association between the MDS and either stroke morbidity or mortality. Data on stroke morbidity was available in the MONICA-Copenhagen cohort only, whereas data on stroke mortality was available in both the MONICA-Copenhagen and the VIP cohorts.

Intervention studies have found that decreased inflammation levels and improved endothelial functions are the potential biological mechanisms that justify the health effects of this dietary pattern (Schwingshackl and Hoffmann, 2014). These mechanisms might explain the effects on CVD prevention that I observed in both the VIP and MONICA-Copenhagen cohorts, although I could not draw any conclusions regarding a protective effect of the Mediterranean diet on the risk of stroke.

Key messages

- Consumption of typical Mediterranean foods protects against CVD.
- The original MDS is calculated using intakes of typical Mediterranean foods, but it does not cover all Mediterranean foods that are protective to CVD (e.g. wholegrain cereals).
- A refined MDS developed to more accurately describe adherence to a typical Mediterranean diet, was found to be inversely associated with the risk of CVD, although not with stroke.

Table 6: Association between the MDS (refined and original version) and CVD incidence and mortality

Cox proportional hazard models were used to calculate HRs and 95% confidence intervals (CIs) estimating the association between a one unit increase in the refined and original MDS and the risk of CVD incidence and mortality (total, MI, and stroke). Stroke included both haemorrhagic and non-haemorrhagic subtypes. The number of participants in each cohort is reported, together with the number cases per cohort per outcome.

	HRs (95% CIs)[1]								
	MONICA-Copenhagen (n = 1,849)						**VIP (n = 73,984)**		
	Total CVD		**MI**		**Stroke**		**Total CVD**	**MI**	**Stroke**
Score version	**Incidence (n = 755)**	**Mortality (n = 223)**	**Incidence (n = 161)**	**Mortality (n = 64)**	**Incidence (n = 167)**	**Mortality (n = 40)**	**Mortality (n = 680)**	**Mortality (n = 305)**	**Mortality (n = 144)**
Original score	0.97 (0.93; 1.02)	0.96 (0.89; 1.05)	1.00 (0.91; 1.01)	0.88 (0.76; 1.02)	0.96 (0.88; 1.06)	1.06 (0.88; 1.29)	-	-	-
Refined score	0.92*** (0.87; 0.97)	0.87** (0.79; 0.95)	0.89* (0.80; 1.00)	0.79** (0.66; 0.94)	0.93 (0.84; 1.04)	0.94 (0.76; 1.17)	0.96 (0.92, 1.01)	0.94 (0.87, 1.01)	0.99 (0.89, 1.09)

[1] From Cox-proportional hazard models adjusted for sex, age, BMI, smoking status, education, and physical activity. CVD = Cardiovascular disease. MI = Myocardial Infarction.
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

5.1.4 Cumulative effects of diet and lifestyle

Lifestyle risk factors often coexist within the same individual and are also associated with a higher risk of other chronic disease such as cancer (Handy *et al.,* 2018) and type II diabetes (Wu *et al.,* 2014). I have shown in the previous section (5.1.3) that the MDS is protective for CVD, but diet is only one of the lifestyle factors linked to the risk of CVD. Low physical activity, cigarette smoking, and obesity all are modifiable lifestyle factors that have a strong cumulative impact on the risk of developing CVD and they need to be taken into consideration to estimate the risk reduction that can be obtained by a healthier lifestyle (Bhatnagar, 2017). Acknowledging that diet is only one component of a healthy lifestyle and that CVD is a multi-factorial disease, the cumulative impact of a series of lifestyle factors on the risk of mortality was explored in both the VIP and the MONICA-Copenhagen studies using a lifestyle score. The latter was calculated by giving an increasing number of points based on a series of diet and lifestyle factors (see 4.6 in method section). In the VIP cohort, the lifestyle score was calculated by giving an increasing number of points based on several healthy lifestyle factors such as having an MDS > 4, being a never smoker, being physically active, and having a BMI < 30 kg/m^2. The results, depicted in Figure 2, showed a cumulative inverse association between an increasing number of healthy diet and lifestyle factors and the risk of mortality. In particular, the HR for the highest vs the lowest score level was 0.38 (95% CI: 0.27, 0.54) in men and 0.38 (95% CI: 0.25, 0.57) in women.

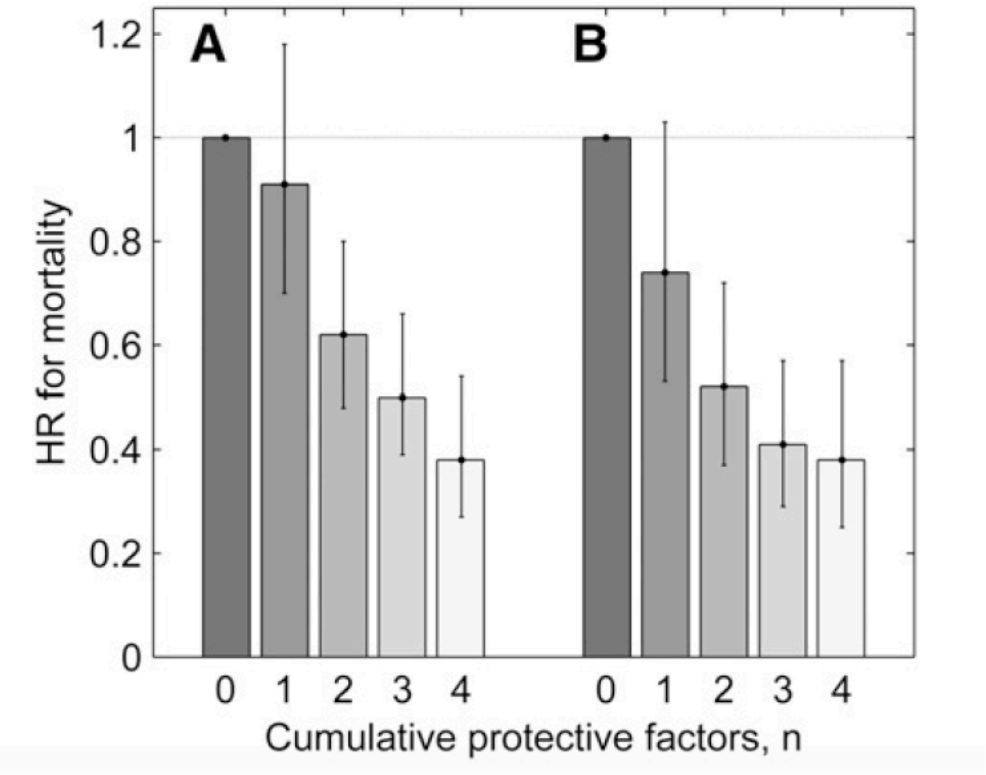

Figure 2: Cumulative association of lifestyle protective factors and all-cause mortality
Cox proportional hazard models were used to calculate age-adjusted HR and 95% CI models testing the association between a cumulative number of protective dietary and lifestyle factors and all-cause mortality, stratified by men (n = 35,950; panel A) and women (n = 38,034; panel B). Hazard Ratios (HRs) refer to the comparison between having no risk factors (0) and having a number of risk factors from 1 to 4. These analyses were conducted in the VIP cohort and adjusted by age only.

In the MONICA-Copenhagen study, the score was instead calculated by giving an increasing number of points based on a series of unhealthy factors such as ever being a smoker, having a BMI > 30 kg/m^2, being sedentary and having a refined MDS < 3. The results showed that an increasing number of unhealthy diet and lifestyle factors were cumulatively associated with stronger mortality risk. The HRs, from Cox proportional hazard models, showed a cumulative direct association between an increasing number of unhealthy diet and lifestyle factors and the risk of mortality. In particular, the HR for the comparison between the highest the lowest risk categories was 2.37 (95% CI 1.50, 3.75; p for trend = 0.0001) (Figure 3).

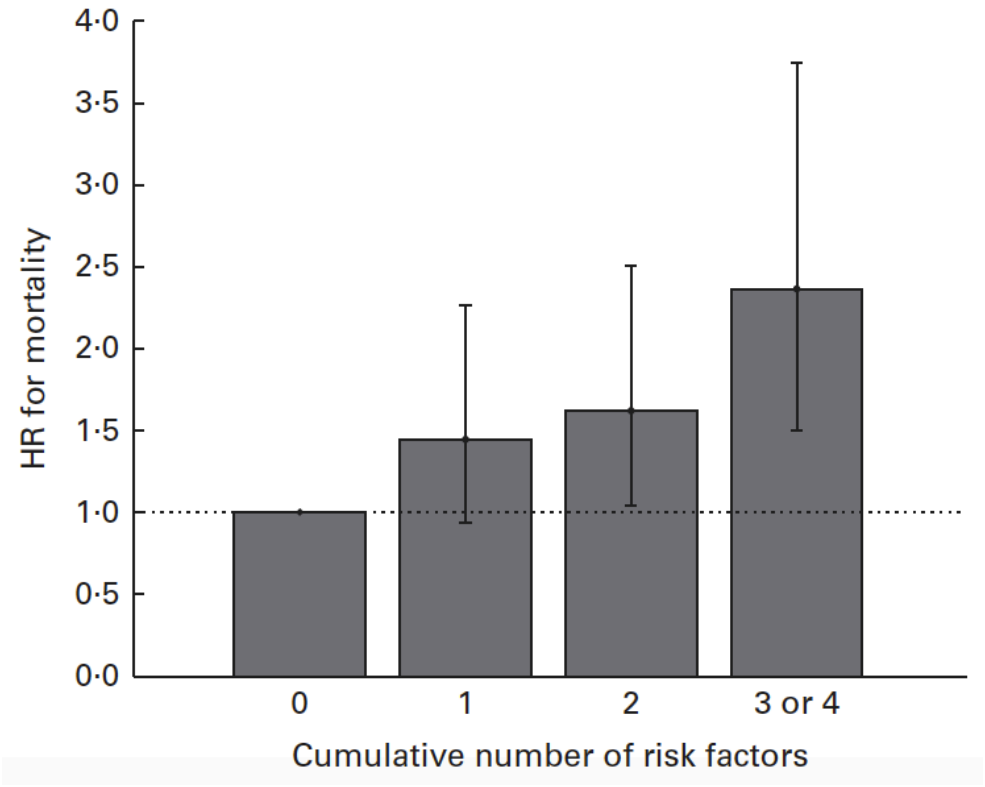

Figure 3: Cumulative association of lifestyle risk factors and all-cause mortality
Cox proportional hazard models were used to estimate HRs and 95% CIs (the latter represented by vertical bars) of the association between a cumulative number of risk factors related to both diet and lifestyle (including a modified MDS ≤ 4, ever being a smoker, low levels of physical activity and being obese) and all-cause mortality (adjusted by age). 144 subjects had no risk factors, 606 had one, 787 had two, and 312 had 3 or 4 risk factors. P for trend < 0.0001.

Key messages

- Diet is only one of the lifestyle factors that are associated to CVD risk.
- Healthy and unhealthy lifestyle factors often coexist within the same individuals and are also associated with other chronic diseases.
- By combining four healthy lifestyles (i.e., high adherence to the Mediterranean diet, normal body weight, abstinence from cigarette smoking, and high physical activity) it is possible to reduce the risk of CVD to less than half.

5.1.5 Sensitivity analyses

One of the major limitations of observational studies links to the difficulty controlling for potential confounding factors and the greater risk of bias compared to randomized controlled trials (Nørgaard *et al.,* 2017). Therefore, it is important to confirm observational results with a series of sensitivity analyses to rule out potential causes of biased or distorted results.

To confirm the results obtained with the analyses described above, I repeated the latter after excluding subjects who died during the first two years of follow-up since their death might have been due to subclinical diseases that were already present at enrolment and not because of incident CVD presenting during the follow up period. These analyses did not produce any material changes in the results. For instance, in the VIP cohort the HR was 0.97 instead of 0.96 with a similar 95% confidence interval (0.94; 1.00), in the MONICA-Copenhagen study the HR and 95% confidence interval was unchanged for total mortality, whereas differences in the rage of max 0.02 units (i.e., HR of 0.90 instead of 0.92) were observed for CVD incidence and mortality.

In addition, I confirmed my results in a sub-sample of "adequate reporters" obtained by excluding potential under-reporting subjects identified using a ratio between food intake levels (FIL) and physical activity levels. The results showed that the MDS was always inversely associated with both morbidity and mortality, although they were not always statistically-significant due to a reduction in the sample size, especially with the most stringent criteria (i.e., > 65% of the ratio between energy intake and basal metabolic rate). Finally, in the H70 study we also observed unchanged results when adjusting for a variable describing activities of daily living and a slight difference in both the HR and the 95%

confidence interval related to the association of the refined score with total mortality (HR = 0.92, 95% CI: 0.86; 1.00).

All the above analyses confirmed my results in all three studies.

5.2 Aim 2: Dairy product intake and mortality

In the second part of this thesis, I aimed to analyse the association between intakes of different types of dairy products and the risk of total mortality. The rationale for conducting these analyses was that, in the MDS calculations, dairy products are considered atypical Mediterranean food products and a homogeneous food group. On the contrary, dairy products are a varied group of food, each of which is produced with specific processing methods that can influence their health properties (Mozaffarian and Wu, 2018). Consumption of dairy products that contain intact milk fat globule membrane (MFGMs), such as non-homogenized milk or yoghurt, and cheese produced with non-homogenized milk for example, are potentially associated with several health benefits compared to foods such as butter and fermented milk, where the MFGMs have been destroyed by with milk processing (Gallier *et al.,* 2014; Ye *et al.,* 2017). On the other hand, although fermented milk products are usually made of homogenised and pasteurised milk, their consumption could also favourably influence a healthier composition of the gut microflora thanks to the fact that they are fermented (Butel, 2014; Linares *et al.,* 2016).

Reactive to evidence that each dairy product has specific characteristics depending on its production process, and some (e.g., cheese and fermented milk) are typical Mediterranean food products, I explored the association between individual dairy products and mortality to understand whether adaptation of the MDS calculation process was necessary to enable assessment of their relative impacts on CVD outcomes. My hypothesis was therefore that

the association between consumption of dairy products was not consistent with regard associations with mortality for all different types, and that only consumption of atypical Mediterranean food products, such as non-fermented milk and butter, was directly associated with a higher risk of mortality. The association between intakes of individual dairy products (i.e., milk, yoghurt, cheese, and butter) with all-cause mortality was investigated in three cohort studies: the VIP (Tognon *et al.,* 2017) and the Northern Sweden MONICA (Tognon *et al.,* 2017), as well as the H70 study (Tognon *et al.,* 2018).

5.2.1 Associations between dairy product intakes and all-cause mortality in the VIP and Northern Sweden MONICA studies

In the calculations of the original MDS, all dairy products are categorised as atypical Mediterranean foods (Trichopoulou *et al.,* 1995). However, as previously argued (section 5.2), it is reasonable to assume that different dairy products would have different health effects, considering the heterogeneity that characterises this food group (Gille *et al.,* 2018). Until recently, nutritional epidemiologists have classified dairy products based on fat content, combining high- and low-fat products irrespective of dairy types, because of a general concern for the health effects imputable to SFA consumption (Lordan *et al.,* 2018). Following a discussion stimulated by Michaëlsson *et al.* (2014) who showed that milk and cheese intakes can have opposite associations with mortality, I decided to investigate more in detail this food group in all cohorts.

The associations between all-cause mortality and intakes of non-fermented milk, fermented milk, cheese, and butter were tested in Cox proportional hazard models adjusted for several potential confounders including age, sex, BMI, screening year,

education, smoking status, and total energy intake (adjusted models). Crude models were only adjusted by age and sex (Table 7).

The inverse association between fermented milk and mortality was statistically significant in the crude model only. This result could be due to the fact that only 28% of participants consumed non-fermented milk on a daily basis, compared to 61% and 46% of participants who consumed milk and cheese, respectively, on a daily basis. All other associations were statistically significant in both crude (i.e., age- and sex-adjusted) and adjusted models, although the estimates were slightly attenuated in the latter. Non-fermented milk and butter were both directly associated with mortality, whereas an inverse association was found for cheese intake. Additional adjustment for physical activity did not affect the results.

Table 7: Association between intakes of different types of dairy product and all-cause mortality

I used Cox proportional hazard models to calculate HRs and 95% CIs for the association between intakes of different types of dairy products and all-cause mortality, adjusted for age and sex (crude models) or further adjusted for BMI, screening year, smoking, education, and energy intake. Energy and intakes of different dairy products were included as reported intakes per day as continuous variables. Both the number of total participants and the number of mortality cases are reported. All estimates refer to an increase in one portion per day of each dairy product.

	HRs by dairy product			
	Non-fermented milk	**Fermented milk**	**Cheese**	**Butter**
N of subjects	101,019	101,019	101,019	101,019
Mortality cases	6,892	6,892	6,892	6,892
	HRs (95% CI)			
Crude models	1.05 (1.03, 1.07)***	0.90 (0.86, 0.94)***	0.93 (0.91, 0.96)***	1.03 (1.01, 1.05)***
Adjusted models	1.02 (1.00, 1.05)*	0.96 (0.92, 1.01)	0.94 (0.91, 0.97)***	1.02 (1.00, 1.04)*

* $p < 0.05$, *** $p < 0.001$.

Key messages

- The methods used in the production of dairy products affect their health properties, both negatively and positively. Therefore production methods should be taken into consideration when assessing the impact of dairy products on health and disease outcomes.
- Investigating the health effects of dairy product intakes adjusting for fat content only does not allow elucidation of potential differences connected to the consumption of different product types.

- Based on my results, both milk and butter intakes predict risk of mortality, whereas cheese intakes suggested a protective effect.
- The association between yogurt intakes and mortality is less certain, although there is a tendency toward an inverse association with mortality. A lower frequency of consumption might contribute to explain this result.

5.2.2 Association of non-fermented milk intakes with all-cause mortality stratified by fat content

High consumptions of dairy products have traditionally been discouraged by official dietary recommendations because of their high saturated fat content (Nordic Council of Ministers, 2012). However, this recommendation has often been criticised, since it is believed that the effect of saturated fats on CVD risk factors, such as total cholesterol levels, differ based on the food product consumed. As an example, it is known that butter intake has a stronger influence on cholesterol levels compared to cheese intake (Feeney *et al.,* 2018). Also, consuming low-fat dairy products only, would lead to a reduced intake of liposoluble vitamins such as vitamin D and K (Lordan *et al.,* 2018).

The majority of saturated fatty acids found in milk (i.e., myristic 14:0, palmitic 16:0, and stearic acid 18:0) are considered atherogenic (Attia *et al.,* 2015), therefore the possibility that dairy fat plays at least a partial role in determining dairy products' health effects cannot be ruled out. Considering the large number of subjects in my cohorts reporting intakes of milk with different fat contents (i.e., low-, medium, and high-fat), I decided to compare exclusive vs. total intakes of non-fermented milk characterised by increasing fat content, such as low (0.5%), medium (1.5%), and high-fat (3%). The results reported in table 8 show that, when analysing the whole cohort, only whole milk intakes predicted mortality in adjusted models. On the other hand, when the analyses were restricted to exclusive drinkers of each type of milk, the HRs of the three types of milk were very similar, although only intakes of medium-fat milk were associated with mortality in adjusted models.

Table 8: Association between milk intakes and all-cause mortality stratified by fat content
I used Cox proportional hazard models to estimate HRs and 95% CIs for the association between milk intakes (stratified by fat content, total or exclusive intakes of each type) and all-cause mortality. Models were adjusted for age and sex (crude models) or further adjusted for BMI, screening year, smoking status, education, and energy intake (adjusted models). Both the number of total participants and the number of mortality cases are reported. Energy intake was included as reported intake/day (continuous variable). Additional adjustment for physical activity with exclusions made for missing information did not affect any HRs. Models including all subjects and missing values as a dummy category only affected the HRs and 95% CIs by 1 unit of the second decimal. All estimates refer to an increase in one portion per day of each milk subtype.

	HRs and 95% CIs by milk fat content		
	High fat (3%)	**Medium fat (1.5%)**	**Low fat (0.5%)**
All subjects			
Participants who reported intake ≥ 1/week	16 183	62 856	24 699
Mortality cases, N (%)	1 551 (9.6)	3 875 (6.2)	1 829 (7.4)
Hazard ratios (95% CI)			
Crude model	1.13 (1.08, 1.18)***	1.05 (1.01, 1.08)**	1.05 (1.01, 1.10)*
Adjusted model	1.08 (1.03, 1.14)**	1.01 (0.98, 1.05)	1.03 (0.98, 1.08)
Subjects with exclusive milk type preference			
Participants (reported intake ≥1/week)	6 177	27 966	6 566
Mortality cases, No (%)	710 (11.5)	1 769 (6.3)	569 (8.7)
Hazard ratios (95% CI)			
Crude model	1.12 (1.05, 1.19)***	1.13 (1.08, 1.19)***	1.09 (1.01, 1.18)*
Adjusted model	1.06 (0.99, 1.13)	1.08 (1.03, 1.14)**	1.07 (0.98, 1.16)

*p < 0.05, **p < 0.01; ***p < 0.001.

Key messages

- Research evidence highlights high probability that relationships between dairy products and health outcomes will be influenced by both fat content and dairy type.
- Milk contains atherogenic SFA: myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids.
- In my results, high-fat milk intakes predicted stronger negative effects compared to medium- and low-fat options, although I could not confirm these results in subjects drinking exclusively one type of milk.

5.2.3 Associations between dairy product intakes and all-cause mortality in the H70 study

The health effects of dairy products in the elderly are particularly controversial. Dairy products are a rich source of protein and calcium, which are recommended for older individuals (Office of the Surgeon General, 2004; Bauer *et al.,* 2013), yet they also contain atherogenic SFA. Therefore, in view of the results obtained in the VIP study, I replicated the same analyses in a cohort of elderly people, the H70 study (Tognon *et al.,* 2018). Unfortunately, due to a constraint linked to the dietary assessment methods used in this study, intakes of individual milk products (i.e., milk, yoghurt, and sour milk) were not available and were therefore combined prior to comparison with cheese.

I found an inverse association between cheese intake and all-cause mortality. The analyses were stratified by follow-up duration because the assumption of proportionality of hazard during the follow-up time was not fulfilled. The results obtained in all four birth cohorts combined showed that the HRs tended to decrease at longer follow-up times indicating a potential period effect since the follow-up was longest in the oldest birth cohort (table 9). On the contrary, milk product intakes were directly associated with mortality, with stable

HRs across increasing follow-up times. The analyses performed after excluding subjects who died during the first two years of follow-up confirmed these results. Similar results were obtained when I estimated missing values for both physical activity and education level by multiple imputation.

Table 9: Association between intakes of cheese and milk products with all-cause mortality, stratified by follow-up duration

Cox proportional hazard models were used to estimate HRs and 95% CIs for the association between cheese (10 g/day) and milk product (non-fermented milk, yogurt, and sour milk, 100 g/day) intakes with all-cause mortality, stratified by follow-up duration. The results on the total follow-up duration were obtained including an interaction term between exposure and follow-up time. Basic models were adjusted for sex and birth cohort (included as stratification variable). Adjusted models were further adjusted for smoking status, BMI, education, marital status, physical activity and total energy intake. Both the number of mortality cases at each follow-up and the total number of participants are reported.

Follow-up duration	Cases/ Tot. subjects	Cheese (10 g/day)		Milk products (100 g/day)	
		Basic model HRs (95% CIs)	Adjusted model HRs (95% CIs)	Basic model HRs (95% CIs)	Adjusted model HRs (95% CIs)
12 years	411/ 1,213	0.93 (0.90; 0.97)***	0.94 (0.91; 0.98)**	1.04 (1.00; 1.08)	1.04 (1.00; 1.08)
20 years	728/ 1,213	0.96 (0.94; 0.99)**	0.97 (0.94; 1.00)*	1.04 (1.00; 1.07)*	1.04 (1.01; 1.08)*
32 years	831/ 1,213	0.96 (0.94; 0.98)**	0.97 (0.94; 0.99)*	1.03 (1.00; 1.07)*	1.04 (1.01; 1.08)**
Total	833/ 1,213	0.91 (0.86; 0.97)**	0.92 (0.87; 0.98)**	1.06 (1.00; 1.13)*	1.06 (1.00;1.12)‡

*p-value < 0.05, **p-value < 0.01, ***p-value < 0.001, ‡p = 0.07.

Key messages

- Dairy products represent a valuable source of protein and calcium, which are important nutrients for elderly people.
- Reactive to the increased risks of CVD observed in elderly groups, advice regarding dairy products is required to enable adequate protein and calcium consumption in the absence of products which are rich in atherogenic SFA.
- Milk product intakes (milk, yogurt, and sour milk) were found to directly predict risk while cheese intake was found to be protective for the risk of mortality in elderly subjects.

5.2.4 Dose-response analyses in the H70 study

With the aim of finding indications of potential causality of the association between dairy product intakes and mortality, dose-response analyses were conducted.

In the combined VIP and Northern Sweden MONICA cohort, high consumers of nonfermented milk (≥ 2.5 times/day) had a 32% increased mortality risk compared with that of subjects consuming milk ≤ 1 time/week (HR = 1.32, 95% CI: 1.18; 1.48). For butter intakes, the increased mortality risk between the lowest and the highest consumption levels was 11% (HR = 1.11, 95% CI: 1.07; 1.21).

Table 10 shows the results of dose-response analyses in the H70 cohort, in which the lowest tertile of both cheese and milk product intakes were compared with the other two tertiles. The HRs were stratified by follow up times as in the main analyses and adjusted for the same potential confounders (i.e., sex, birth cohort, smoking status, BMI, education, marital status, physical activity, and total energy intake). The association between cheese and mortality was stable across tertiles, failing to show a dose-response association. On the

contrary, a dose-response association was found for milk products, but only at the longest follow-up. This suggests that the inverse association between cheese intake and mortality was less likely to be causal and is possibly explained by residual confounding by healthy lifestyle factors associated with high cheese intakes, such as lower meat intakes.

Table 10: Dose-response association between cheese and milk product intakes with all-cause mortality

Results from Cox proportional hazard models testing the association between intakes of cheese (10 g/day) and milk products (non-fermented milk, yogurt, and sour milk, 100 g/day) with total mortality, across increasing sex-specific intake tertiles and stratified by follow-up durations. All models were adjusted for sex, birth cohort (included as a stratification variable), smoking status, BMI, education, marital status, physical activity, and total energy intake. Both the number of mortality cases at each follow-up and the total number of participants are reported.

Follow-up duration	Cases/Tot. subjects	HR (95% Confidence intervals)			p for trend
		Low	Med	High	
		Cheese (10 g/day)			
12 years	411 / 1,213	1	0.87 (0.69; 1.09)	0.80 (0.61; 1.03)	n.s.
20 years	728 / 1,213		0.92 (0.77; 1.10)	0.92 (0.76; 1.12)	n.s.
32 years	831 / 1,213		0.92 (0.78; 1.08)	0.89 (0.74; 1.07)	n.s.
		Milk products (100 g/day)			
12 years	411 / 1,213	1	1.03 (0.80; 1.32)	1.08 (0.84; 1.39)	n.s.
20 years	728 / 1,213		0.98 (0.82; 1.19)	1.17 (0.97; 1.42)	n.s.
32 years	831 / 1,213		1.09 (0.91; 1.29)	1.20* (1.00; 1.44)	< 0.05

n.s. = not significant. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001; †p = 0.06; n.s.: not significant.

Key messages

- A dose-response relationship observed between an exposure and an outcome is an indicator of a possible cause-effect relationship.
- In the H70 study, milk product intakes (i.e., milk, yogurt, and sour milk) showed a dose-response association with mortality.
- Cheese intake did not show a dose-response association with mortality. In the H70 cohort its association with mortality is less strong compared to milk products.

5.2.5 Sensitivity analyses

A series of sensitivity analyses were run to exclude the possibility that the inverse association observed between cheese consumption and mortality was explained by the fact that cheese consumers had a healthier diet compared to the rest of the cohort. Analyses were adjusted for the Healthy Diet Score (see section 4.4), as well as for vitamin D and calcium intakes. Since unfavourable health effects of high galactose intakes have been proposed to explain the direct association of milk intake and mortality (Michaelsson *et al.,* 2014), I also adjusted the analyses for lactose, as a proxy for galactose intake. Finally, I confirmed the results after excluding subjects reporting extreme intakes of dairy products, as well as those subjects who died during the first two years of follow-up, to exclude confounding due to the presence of subclinical disease at recruitment.

All the above analyses produced both HRs and 95% confidence intervals that were either slightly similar or unchanged compared to the ones reported in the main analyses.

5.3 Aim 3: MDS and overweight during early life

It is well known that CVD risk starts before birth (Kuh *et al.,* 2003). Therefore, after exploring the association of the MDS with CVD and mortality in adults, I analysed the association of adherence to a Mediterranean-like diet with overweight and obesity, the two most relevant predictors of the future risk of CVD during childhood (Lloyd *et al.,* 2012). Data was obtained from the multi-country IDEFICS study, a large childhood cohort that recruited thousands of children in 8 European countries (Ahrens *et al.,* 2011). The latter included both Mediterranean (Italy, Spain, and Cyprus) and non-Mediterranean countries (Sweden, Germany, Belgium, Hungary, and Estonia).

5.3.1 Adherence to the Mediterranean diet across countries

A progressive abandonment of the Mediterranean diet among young individuals had been highlighted in previous studies conducted in South Europe (Cabrera *et al.,* 2015). Among the factors used to justify this include reduced time and attention for both food purchasing and cooking, greater consumption of processed foods, and a consequent excessive intake of foods containing cholesterol, saturated fats, and refined carbohydrates (Serra-Majem *et al.,* 2001). A recent systematic review summarised the results from 58 observational and intervention studies which investigated adherence to a Mediterranean-like diet in children and adolescents (Iaccarino *et al.,* 2017). The results showed large variations in adherence among European countries, although only a few studies were conducted in non-Mediterranean countries.

The IDEFICS cohort gives the opportunity to investigate the distribution of high adherence to a Mediterranean-like diet across Europe. Surprisingly, the greatest proportion of high adherent children was found in the Swedish cohort (Table 11). In particular, 56.7% of

Swedish children had high fMDS levels (> 3), followed by Italian (37.5%) and German children (35.1%). Swedish children reported the highest (presented as % sample with intakes greater than the median) consumption of vegetables (81.2%), fruit and nuts (73.4%), as well as grains (74.2%), whereas, unexpectedly, Italian children had the lowest consumption levels of vegetables (28.6%, Table 11). It is important to underline that the Swedish families had the highest socioeconomic levels in the entire study. Although these analyses were adjusted by socioeconomic status, the presence of residual confounding could therefore partly explain these paradoxical results (Ahrens *et al.,* 2011).

Table 11: Distribution of consumption of typical and low consumption of atypical Mediterranean food groups

Children characterised by intakes above or below sex- and age-specific median intakes of each food group included in the fMDS calculations. The percent of children with high fMDS (> 3) is shown together with the differences (and 95% confidence intervals) between the fMDS in each country and Sweden. The total number of children per cohort is also shown.

Food groups	Country where the survey centre was conducted							
	Sweden (n = 1744)	**Italy (n = 2200)**	**Germany (n = 1979)**	**Spain (n = 1460)**	**Hungary (n = 2486)**	**Belgium (n = 1820)**	**Estonia (n = 1644)**	**Cyprus (n = 1637)**
Percent of subjects with intakes above the sex- and age-specific median intake of:								
Vegetables	81.2	28.6	64.7	40.5	51.7	68.6	48.1	37.9
Fruit & nuts	73.4	57.5	49.3	67.7	52.3	51.2	62.4	65.2
Cereals	74.2	50.2	47.1	49.7	51.3	44.9	63.1	49.1
Fish	68.4	75.3	34.0	82.2	30.2	49.1	47.1	39.0
Percent of subjects with intakes below the sex- and age-specific media of:								
Dairy products	38.9	53.0	50.4	28.0	49.3	55.2	44.2	47.2
Meat products	88.4	88.2	93.3	87.2	94.0	83.6	65.5	75.3
Proportion of children with high adherence to the Mediterranean diet								
fMDS > 3	56.7	37.5	35.1	31.8	32.2	32.7	26.3	24.2
Difference (95% CI) with fMDS in Sweden								
fMDS	(4.2) (Ref.)	- 0.68 (-0.58; -0.47)	-0.70 (-0.60; -0.49)	-0.80 (-0.68; -0.56)	-0.81 (-0.71; -0.61)	-0.85 (-0.74; -0.63)	-1.03 (-0.92; -0.81)	-1.16 (-1.03; -0.90)

Children from Cyprus were characterised by the lowest adherence levels: approximately 75% of them had a fMDS ≤ 3. A statistically significant difference between centres was detected by ANOVA ($p < 0.0001$). The score difference between the highest (i.e., Swedish children) and lowest adherent participants (i.e., Cypriot children) was 1.16 (95% CI: 1.03; 0.90). Figure 4 shows the ecological distribution of fMDS which indicates no clear geographical pattern (e.g. north-to-south gradient).

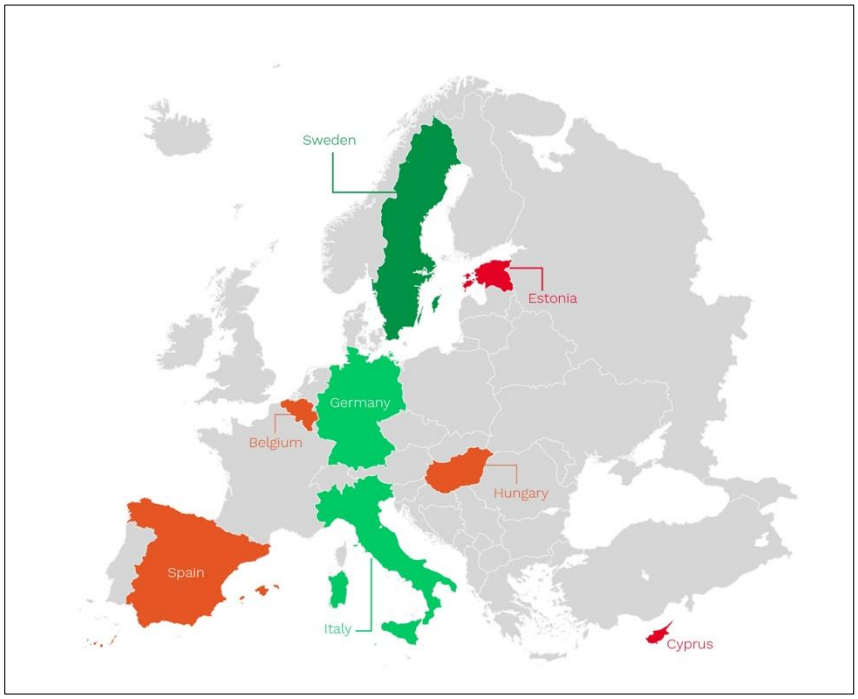

Figure 4: Distribution of high adherent children across IDEFICS countries
Ecological distribution of high (> 3) fMDS across the centres included in the IDEFICS study. Sweden (dark green tone) was the country showing the highest adherence levels, followed by Germany and Italy (semi-dark green tone), Belgium, Spain and Hungary (light red tone) and finally by Estonia and Cyprus (dark red tone).

Key messages

- A reduction in adherence to the Mediterranean diet has been reported among young individuals living in South Europe.

- The majority of studies on the Mediterranean diet have been conducted in South Europe, therefore there is a scarcity of data on adherence in populations living outside its region.
- The results confirmed that adherence levels are low in other European countries (and lowest in Cyprus), although Swedish children had the highest adherence.

5.3.2 Inverse association between the fMDS and several outcomes in children

Observational studies in adults showed an inverse association between the frequency-derived MDS score and lower waist circumference and therefore a reduced risk of central obesity (Romaguera *et al.,* 2009), as well as with a reduced risk of metabolic syndrome (Kesse-Guyot *et al.,* 2013), type II diabetes (Martínez-González *et al.,* 2008; Koloverou *et al.,* 2014), and (more in general) with a healthier body weight (Buckland *et al.,* 2008; Shai *et al.,* 2008). The association between adherence to the Mediterranean diet and childhood obesity is less clear (Iaccarino Idelson *et al.*, 2017).

To determine whether adherence to a Mediterranean-like diet was inversely associated with the risk of overweight and obesity in children, I tested the association between the fMDS and different indicators of overweight and obesity, both cross-sectionally and longitudinally. Cross-sectional results showed that high fMDS (i.e., > 3) were inversely associated with potential mediators of the association between diet and CVD such as overweight and percent fat mass. In contrast, no association was found between adherence to a Mediterranean-like diet and waist circumference or WtHR (Tables 12 and 13).

Table 12: Cross-sectional association between the fMDS and the prevalence of overweight and obesity and percent fat mass

Cross-sectional association, from logistic (overweight) and linear regression (percent fat mass) analyses, between high adherence to a Mediterranean-like dietary pattern (i.e., fMDS > 3) and indicators of overweight. Fully-adjusted models included sex, age, study centre, as well as parental education and income.

	OR and 95% CIs	
	Overweight including obesity	**Percent fat mass**
Crude (unadjusted) models	0.83***	-0.42**
	(0.76; 0.91)	(-0.70; -0.14)
Fully-adjusted models	0.85**	-0.22*
	(0.77; 0.94)	(-0.43; -0.01)

* = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Table 13: Cross-sectional association between the fMDS, WtHR and waist circumference

Cross-sectional associations, from logistic regression analyses, between fMDS > 3 and indicators of overweight. Crude models were unadjusted in cross-sectional analyses but adjusted for baseline WtHR or waist circumference in longitudinal analyses. Fully-adjusted models included sex, age, study centre, parental education and income.

	Odds ratios and 95% Confidence Intervals	
	WtHR > 0.5	**Waist circumference**
Crude (unadjusted) models	0.92	-0.48**
	(0.83; 1.02)	(-0.76; -0.20)
Fully-adjusted models	1.00	-0.20
	(0.89; 1.12)	(-0.46; 0.06)

Key messages

- Evidence from previous studies does not clearly establish whether adherence to the Mediterranean diet could contribute to a reduction in childhood obesity.
- I found a cross-sectional inverse association between high adherence levels (fMDS > 3) and overweight and obesity prevalence in young children aged 2-9 years.

5.3.3 Longitudinal analyses

Childhood obesity and rapid weight gain early in life predict cardiovascular health and premature death later in life. Since the aetiology of childhood obesity is still largely unclear however, a better understanding of the predictors of weight gain in children could potentially favour earlier detection of children at risk as well as more targeted interventions (Bichteler and Gershoff, 2018).

The longitudinal analyses in the IDEFICS study tested the association between baseline fMDS > 3 and the highest quintiles of changes in BMI z-scores, waist circumference and WtHR, and % fat mass in logistic regression models. The latter were adjusted for sex, age, socioeconomic status (i.e., parental education and high income), and study centre. One variable describing the baseline value of each outcome (i.e., BMI z-score, waist circumference, etc.) was also included as a covariate in each model. All associations were statistically significant, exception made for % fat mass which was borderline significant ($p = 0.06$) (Table 14). Adjustment for a variable describing inclusion in the intervention study did not modify the estimates.

Table 14: Association between fMDS at baseline and change in overweight indicators after 2 years

I used logistic regression to calculate odds ratios (ORs) and 95% CIs of the association between high adherence to a Mediterranean-like diet (i.e., fMDS > 3) and the highest quintile of change (from baseline to follow-up) in selected indicators of overweight and obesity such as BMI z-score change, waist-to-height ratio (WtHR), waist circumference change and percent fat mass. Fully-adjusted models included sex, age, study centre, as well as parental education and income. In contrast, crude models were only adjusted for the baseline value of each anthropometry variable analysed (e.g., BMI, WtHR, etc.). The total number of children included in each analysis is shown and slightly varies because data on specific measurements (i.e., waist circumference and skinfold measurements) were not available for all children.

	Highest quintile of			
	BMI z-score change (n = 9,196)	**WtHR change (n = 8,796)**	**Waist circumference change (n = 8,796)**	**Percent fat mass change (n = 8,387)**
Crude models	0.81*** (0.73; 0.90)	0.82*** (0.73; 0.91)	0.82*** (0.73; 0.91)	0.86** (0.77; 0.96)
Fully-adjusted models	0.87* (0.78; 0.98)	0.88* (0.78; 0.99)	0.87* (0.77; 0.98)	0.89[a] (0.78; 1.00)

* = p < 0.05; ** = p < 0.01; *** = p < 0.001. [a]p =0.6

Key messages

- Gaining an improved understanding of the predictors of weight change early in life could contribute to knowledge on the aetiology of childhood obesity, enabling improved identification of high-risk children and more targeted interventions among the latter.
- An inverse association between high adherence to a Mediterranean diet in children and a 2-year change in BMI, waist circumference, WtHR, and (although borderline significant) % fat mass, was identified in these analyses.

- Increasing adherence to the Mediterranean diet in children could help prevent excessive weight gain in young children.

5.3.4 Centre-specific analyses in the IDEFICS study (cross-sectional and longitudinal)

The above analyses were conducted on the entire IDEFICS dataset, including data on boys and girls living in 8 EU countries. Aiming to confirm the above results association in each study centre separately, I tested the association between the fMDS and overweight (including obesity) stratified by centre, both cross-sectionally and longitudinally. No association between fMDS > 3 and overweight (including obesity) was found, either cross-sectionally or longitudinally. In the latter analyses, the association between fMDS > 3 and the highest quintile of change in BMI z-scores at 2 years was tested (Figure 5). The absence of statistically significant associations when stratifying by countries is probably due to calculations of the MDS using sex- and age-specific intake medians for all cohorts combined and not separately in each country.

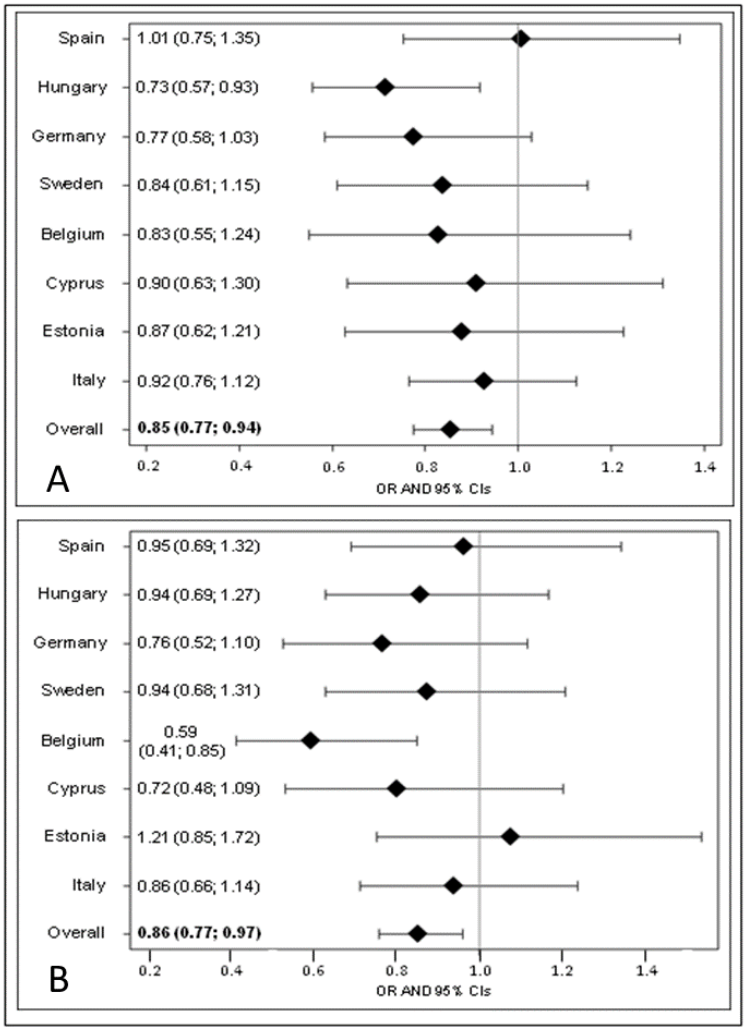

Figure 5: Association between fMDS and overweight and obesity stratified by study centre

Centre-specific (and combined) OR and 95% CIs of the association between high adherence to the fMDS and overweight (including obesity, panel A) as well as the highest quintile of change at follow-up in BMI z-score (panel B).

Key messages

- Country-stratified analyses did not reveal any statistically-significant association between the fMDS and the prevalence of overweight and obesity, possibly due to lack of statistical power.

5.3.5 Sensitivity analyses to confirm the results obtained from CEHQ-FFQ data

Several sensitivity analyses were conducted to confirm the cross-sectional and longitudinal results described above. Since a parent from another country can influence the dietary habits of the whole family, the first analysis was the adjustment for having at least one immigrant parent. In addition, I adjusted for physical activity, which was not included in the main model and which was assessed as the average number of hours spent playing outdoors, or the number of days spent in sports clubs every week. This adjustment helped me understand that energy expenditure did not affect the results. Since children's diet was reported by parents, I also adjusted for the number of meals consumed at home, as a proxy for the number of meals under parental supervision. This analysis was necessary because school meals were not available in all countries or all ages. These adjustments confirmed both cross-sectional and longitudinal results. In cross sectional analyses, results were confirmed to be non-statistically-significant for both WtHR and waist circumference, whereas for the association between the MDS and overweight including obesity the results produced an HR of 0.85 but 95% confidence intervals varied slightly between (0.76; 0.95) and (0.77; 0.94). Larger variations were observed for the percent fat mass where beta estimates varied between -0.16 (95% CI: -0.31; -0.01) and -0.25 (95% CI: -0.48; -0.02). Small variations in both HR and 95% confidence intervals were observed for longitudinal analyses. More details about these results have been given in supplementary tables 1 and 2 in Tognon et al. (2014a).

The longitudinal association with % fat mass became statistically significant after adjustment for physical activity (OR = 0.88, 95% CI: 0.76; 0.98, p = 0.045). Exclusion of children characterised by the highest 5% of BMI, waist circumference, WtHR or % fat mass (according to which outcome was analysed) also confirmed all results.

5.3.6 Comparison of adherence levels using 24HDR

Since food practices can be strongly influenced by the external environment, school meals are considered strategic for the promotion of healthier eating habits and constitute a significant portion of young children's diets (Story *et al.,* 2008). In IDEFICS, FFQs only assessed dietary habits under parental control, whereas 24HDRs also included information regarding foods consumed during school meals. Table 15 compares the prevalence of high adherent children (i.e., MDS > 3) calculated from either the FFQs or the 24HDRs. Although the latter were represented by one single recall, they also included meals consumed at school, whereas the FFQ was filled in by parents only and referred to food under parental control only. It is interesting to note that adherence to a Mediterranean dietary pattern was low in most countries when assessed using either dietary assessment methods, showing that including meals consumed at school do not necessarily have a positive impact on adherence to this pattern. Although the Swedes appeared to be the highest adherent children when using FFQ data, the Italians were the highest adherent children when using SACINA data. This difference could be due to the food served during school meals. School meals in the Italian centre were available at the primary school only where it is common to serve a first course, usually grain-based (such as rice, pasta, or barley), and a second course (usually proteins, including fish and legumes). These two courses are served with vegetables and a piece of fruit. In the schools where the Swedish cohort was recruited, it is common to have a main dish (usually proteins, including fish) plus a buffet with vegetables, legumes, and grains where children can independently decide what they want to eat.

Table 15: Prevalence of high adherent children by study centre
In the IDEFICS study two dietary assessment methods were used: a Food Frequency Questionnaire (CEHQ-FFQ) and a 24h dietary recall (SACINA). The prevalence of high adherence to a Mediterranean-like diet was assessed by calculating the percent of children whose MDS was greater than 3 when calculated with data from either dietary assessment methods (i.e., CEHQ-FFQ and 24HDR).

Dietary assessment	% High adherent children (fMDS > 3)							
	Sweden	Italy	Germany	Spain	Hungary	Belgium	Estonia	Cyprus
CEHQ-FFQ	56.7	37.5	35.1	31.8	32.2	32.7	26.3	24.2
SACINA (24HDR)	37.6	49.3	29.4	34.3	34.3	35.5	33.0	29.6

Key messages

- School meals constitute an important proportion of children's dietary intakes.
- The results in 24HDRs (which also include school meals) confirmed that the adherence to the Mediterranean diet is low among European children as previously found with FFQ data, which only including foods consumed under parental control.

5.3.7 Cultural variance between families with foreign-born vs. native parents

As mentioned above, the presence of one foreign-born parent in a family is likely to influence children's diets. To explore this hypothesis, I ran some analyses and determined that children with at least one foreign-born parent were more likely to have higher fMDS (Table 16). With the exception of Estonian children, the results showed that the prevalence of high-adherent children was higher among those who at least one migrant parent. The latter were often born in a Mediterranean country (i.e. Greece, Croatia, Turkey, France, Morocco) or in a country with a food culture rich in grains, fish, fruit and vegetables (e.g. Ecuador, middle East).

Table 16: Prevalence of high adherent children by parental migrant status

The table below shows the prevalence of high adherence to a Mediterranean-like diet in the IDEFICS study, assessed as the percentage of children whose MDS was greater than 3 calculated from 24HDRs. Study centres are presented according to the geographical location of the countries where they were located: Northern, Central, and Mediterranean Europe. Prevalence of high adherence was stratified based on having native parents or having at least one foreign parent. The percent of children belonging to the latter group is also reported as well as the two most common nationalities among mothers and fathers.

Parental migrant status	**% high adherence (fMDS > 3)**							
	Northern Europe		**Central Europe**			**Mediterranean Europe**		
	Sweden (910)	**Estonia (640)**	**Hungary (961)**	**Belgium (287)**	**Germany (1,265)**	**Italy (1,385)**	**Spain (411)**	**Cyprus (879)**
Native parents	35.7	33.1	34.2	34.6	28.7	48.2	33.7	28.3
At least one foreign parent	46.7	23.3	36.2	43.8	31.2	54.5	36.4	30.0
% children with at least one foreign parent	**14.7**	**22.6**	**5.0**	**6.1**	**33.9**	**16.5**	**10.1**	**30.6**
Most common nationalities (mothers)	Iraqi, Iranian	Russian	Romanian, Croatian	None in particular	Turkish, Kazakh	Swiss, German	Ecuadorean, French, Moroccan	Greek, Georgian
Most common nationalities (fathers)		Russian, Swedish					Ecuadorean, Moroccan	Georgian, Greek

From a public health perspective, children with parents who are foreign-born represent an interesting population group. Integration with the host country is known to amend these subjects' dietary habits, and therefore can also influence health outcomes both positively and negatively (Gilbert and Khokhar, 2008). For instance, some foreign-born subjects (e.g., Asian people) are known to have an increased risk of both obesity and several chronic diseases after moving to Europe (Holmboe-Ottesen and Wandel, 2012). Migration to another country is usually associated with a change in the type of food that it is consumed, although some cultures might be more resistant to change than others, especially those in which the older generations remain part of the close family. Typically, consumption of food items rich in salt, sugar, and fat increases after moving to another country and this depends on several factors, such as convenience, income, availability, and others (Devine *et al.,* 1999). My results are, therefore, unexpected because they seem to contradict this paradigm. However, the IDEFICS dietary data was not sufficiently detailed to capture all dietary patterns potentially associated with a lower obesity risk, such unrefined grain consumption. In addition, the fMDS does not capture the consumption of food products high in sucrose.

Key messages

- The presence of a parent born in another country can sensibly influence the children's diet.
- Previous studies show that migration to another country is often associated with a progressive adoption of unhealthy dietary practices that are common in the new country of residence, although some population can be more resistant to this change.

- By showing high adherence to the Mediterranean diet among children with at least one foreign-born parent, my results contradict the above paradigm. However, I could not determine whether this result was driven by some specific nationalities, since I could not stratify by the country of origin.

6. Discussion

This thesis showed that a refined version of the MDS had a protective association with the risk of total mortality in adult subjects living in Scandinavia, including the elderly. The score refinement consisted in the inclusion of ingredients from recipes and mixed dishes to adapt the score to the local culture. Also, for a more accurate epidemiological contextualization of the Mediterranean diet, I used intakes of food groups that more specifically belong to a genuine Mediterranean diet (e.g., wholegrain cereals instead of total cereals, wine instead of total alcohol intake). The refined score was also inversely associated with both CVD incidence and mortality, excluding stroke incidence and mortality. The latter result was unexpected since a Mediterranean-like diet can reduce risk factors for stroke risk, such as insulin resistance, inflammation, thrombosis, endothelial function, and oxidation (Ding and Mozaffarian, 2006). Consequently, high intakes of typical Mediterranean foods, such as fruit and vegetables (Hu *et al.,* 2014), fish (Xun *et al.,* 2012), and olive oil (Martínez-González, et., 2014), are expected to inversely predict stroke risk. In contrast, high intakes of processed meat (a type of food that is typical of Mediterranean countries, although traditionally consumed in small amounts) are directly associated with stroke risk (Kaluza *et al.,* 2012).

The results in the H70 elderly study showed the importance of a Mediterranean-like diet in reducing the risk of mortality in later life, highlighting that this diet can have beneficial effects across the entire lifespan. Other studies have shown that, by reducing the risk of the most common chronic diseases, a Mediterranean-like diet can favourably influence the ageing process (Trichopoulou *et al.,* 2015).

No intervention trial to date has compared subjects eating a Mediterranean diet with subjects who eat a different diet. Consequently, existing evidence from intervention trials

is weak (Dinu *et al.,* 2018). According to the most recent Cochrane review on the Mediterranean diet (D'Alessandro *et al.,* 2015), many trials focused only on one or two typical Mediterranean foods (e.g., olive oil), rather than the composite pattern of dietary intake. No well-designed RCTs that have investigated all the components of the Mediterranean diet currently exist. The largest trial published so far (i.e., the PREDIMED study) focused on only two food items (i.e., olive oil and nuts), and findings were retracted because of selection bias due to irregularities in the randomisation process. The results have shown the importance of a better contextualization of the epidemiological representation of a typical Mediterranean diet, such as wholegrain cereals and wine, but potentially also typical Mediterranean dairy products, such as cheese and yoghurt.

The second part of this thesis described the association between intakes of different types of dairy products and the risk of mortality in the VIP, Northern Sweden MONICA, and H70 studies. The results showed that cheese intake was inversely associated with the risk of mortality, whereas milk intake (or milk product intake in the H70 study) was directly associated with this outcome. The results on milk intake are in line with those previously found by a Swedish study, which demonstrated that cheese intake was inversely associated with the risk of CVD, cancer, and total mortality (Michaëlsson *et al.,* 2014). However, studies from other countries show a neutral association between milk intake and the risk of CVD and mortality (Guo *et al.,* 2017).

Previous research has not reported any direct association between cheese consumption and mortality or CVD (Guo *et al.,* 2017). My findings highlight that cheese intake is inversely associated with mortality in two studies, corroborating the idea that cheese consumption is not detrimental to health. However, the results on dairy products were not as consistent

as those I obtained for the refined MDS. This inconsistency may be explained the fact that, in the H70 study, the association between cheese intake and mortality was less robust than that observed in the VIP and MONICA studies. Therefore, this means I cannot rule out any possible residual confounding due to any unmeasured factors or to differences in dietary assessment between the two studies (i.e., FFQs vs diet histories). However, cheese represents a heterogeneous food group, and it is possible that the specific type of cheese consumed could determine the consequent health effects. For instance, fermented types of cheese could positively influence the gut microflora, whereas non-fermented cheese products might not have the same effect. On the other side, aged cheese might be richer in peptides than fresh cheese products (Korhonen and Pihlanto, 2006). A recent study found that high cheese intake was inversely associated with the risks of both total and CVD mortality (Farvid *et al.,* 2017). Few previous studies on cheese intake exist and no direct associations with the risk of mortality have been identified. These studies include the Whitehall II (Soedamah-Muthu *et al.,* 2013), the Hoorn (van Aerde *et al.,* 2013), and the EPIC-Netherland study (Praagman *et al.,* 2015). Therefore, my results strengthen existing evidence showing that cheese intake appears to have a neutral (or slightly favourable) association with the risk of mortality.

Unfortunately, I could not draw any firm conclusions regarding fermented milk product intakes, such as yogurt and sour milk (results in the VIP/MONICA studies were not statistically-significant). Also, in the H70 study I could not study fermented milk intakes separately from milk intakes. However, non-significant results warrant further investigation since the association between different types of dairy products and mortality may not be linear and may be driven by very low or very high intakes or u-shaped curves.

Since dairy products are nutrient-rich foods, public health guidelines require careful readdress to ensure they are not excluded from diets, thereby compromising capacity to benefit from the proven favourable health effects of some, due to established connections between this food group and high saturated fat content. This is the reason why I adjusted the statistical models, in sensitivity analyses, for a healthy eating index showing that my results were not modified by diet quality.

Intervention studies have shown that consumption of dairy foods has favourable health effects. The latter include the improvement of the lipid profile (low-fat dairy products only) (Kai *et al.,* 2014), a reduction in both blood pressure (Conway *et al.,* 2014; Drouin-Chartier *et al.,* 2015), and insulin resistance (Tremblay and Gilbert, 2009). This suggests important biological mechanisms linked to cheese consumption could reduce the risk of mortality. However, it is equally possible that cheese consumption simply correlates with other healthy habits.

A surprising result was that, in the VIP and the Northern Sweden MONICA studies, yogurt intake was found to have only a borderline significant association with mortality. Fermented dairy products and probiotic bacteria have both shown to have several positive health effects, including reduction of blood cholesterol and blood pressure as well as improvement of immune function (Biong *et al.,* 2004; Parvez *et al.,* 2006). The results could depend on the fact that the beneficial effects of yogurt could have been masked by the impossibility to distinguish natural from sweetened yogurt, considering that added sucrose has been linked to a higher CVD risk (DiNicolantonio and OKeefe, 2017). Therefore, future studies should analyse natural yogurt intakes separately from other types.

The third and last part of this thesis described the association between the MDS and childhood overweight and obesity, i.e., early risk factors for CVD (Kuh *et al.,* 2003). Paradoxically, Swedish children showed the highest MDS levels, whereas children from Cyprus showed the lowest levels. In general, in all countries but Sweden, the prevalence of high adherent children was below 40%. The MDS was inversely associated with the risk of childhood overweight and obesity (a marker for future CVD risk), both cross-sectionally and longitudinally. Although the IDEFICS study did not recruit a representative sample of European children and, therefore, my results cannot be generalised, they are in line with previous studies that showed low adherence levels to the Mediterranean diet in Europe (Cabrera *et al.,* 2015).

My results in children further reinforce the mounting evidence on the importance of focusing on early CVD risk factors (Kuh *et al.,* 2003), such as childhood obesity, an early cardiometabolic risk factor (Chung *et al.,* 2018). Despite the wide recognition of the health effects of the Mediterranean diet (Katz and Meller, 2014; Dinu *et al.,* 2018), this diet is being abandoned in Mediterranean countries, especially among young people (FAO, 2015). This phenomenon is particularly marked in Southern and Eastern Mediterranean (i.e., North Africa and former Yugoslavia), where a nutrition transition is taking place with undernutrition coexisting with overweight and obesity. In many Mediterranean countries, the traditional diet is being replaced by a diet high in sucrose and saturated fats, and low in fibre, often as a consequence of a transition to a higher socio-economic status. Even in the richest Mediterranean countries, consumption of foods high in animal fat and refined carbohydrates are on the rise (FAO, 2015). The reduced prevalence of people following this dietary pattern is a potential cause of malnutrition since subjects eating a Mediterranean-like diet has been shown to more likely fulfil their dietary requirements (Serra-Majem *et*

al., 2009; Castro-Quezada *et al.,* 2013). There is therefore a strong need for more educational campaigns, especially among young subjects, for promoting a greater adherence to the Mediterranean diet.

6.1 Implications for official dietary recommendations

By showing that the Mediterranean diet (which is rich in fruit, vegetables, nuts, and fish) can prevent CVD and mortality and that dairy product consumption is not necessarily associated with a higher risk of mortality, this thesis has revealed two apparently contradictory sides of the same coin. On one side, that a diet rich in unsaturated fats and vegetable food, but poor in animal food products, is associated with a reduced risk of mortality and chronic disease. On the other side, that foods rich in saturated fats do not necessarily have detrimental health effects, as seems to be the case for some dairy products. In practice, things are more complicated since it is impossible to consume unsaturated fats without SFA. As an example, mackerel, which is considered a source of healthy fats, contains double the amount of total fat and 50% more SFA than red meat. Despite the complexity of this topic, to reduce SFA intake to prevent CHD has become one of the most influential (though debated) dietary advice, despite representing an over-simplification of a complex topic. Most official dietary guidelines worldwide discourage consumption of SFA-rich foods (Nordic Council of Ministers, 2012; USDA *et al.,* 2015; Harcombe, 2019). However, no evidence from observational studies supports the reduction of SFA intakes to prevent future CHD risk (Siri-Tarino *et al.,* 2010; Chowdhury *et al.,* 2014; De Souza *et al.,* 2015). Many observational studies do not investigate the effects of replacing saturated fats with either unsaturated fats or carbohydrates. This replacement was studied only by the Health Professional Study (Zong *et al.,* 2016) and the Nurses' Health

Study (Li *et al.,* 2015), which found no effect when replacing SFA with carbohydrates. In contrast, both studies showed a risk reduction when replacing SFA with PUFA, MUFA, or high-quality carbohydrates. In particular, a comparison of high vs. low intakes of specific saturated fatty acids (i.e., 12:0, 14:0, 16:0, 18:0) showed a risk reduction of 18% CHD cases (Li *et al.,* 2015).

The evidence from clinical trials is more difficult to interpret. Several recent systematic reviews of intervention studies have investigated, by meta-analyses of published trials, the association between SFA intake and the risk of CHD. Some of these found a significant or nearly-significant inverse association between either SFA intake or the replacement of SFA with PUFA and CHD risk (Mozaffarian *et al.,* 2010; Hooper *et al.,* 2015; Sacks *et al.,* 2017). Other systematic reviews did not find similar associations (Schwingshackl and Hoffmann, 2014; Harcombe *et al.,* 2016; Hamley, 2017). A recent review (Hamley, 2017) performed a critical evaluation of possible reasons that could justify these incongruences. One notable reason that explains the apparently conflicting conclusions of the above systematic reviews is that none of them took into consideration a series of nutritional factors that could have confounded the associations between SFA intake and CHD risk in the meta-analyses of the intervention trials they included. For instance, in the LAVAT trial, subjects with higher SFA intakes also had lower vitamin E intakes (Dayton *et al.,* 1968). In addition, the control group in the FMHS trial received a higher dose of cardiotoxic medications (Miettinen *et al.,* 1972), and none of the systematic reviews that found SFA intake to promote CHD discussed the differences in trans-fatty acid intakes between control and intervention groups (Mozaffarian *et al.,* 2010; Schwingshackl and Hoffmann, 2014; Hooper *et al.,* 2015; Harcombe *et al.,* 2016; Hamley, 2017; Sacks *et al.,* 2017). By separately analysing intervention trials that adequately controlled for possible confounders and those that did

not, Hamley (2017) concluded that almost all systematic reviews that found a reduction in CHD events associated with the replacement of SFA with n-6 PUFA, included inadequately controlled trials.

Another relevant factor that helps explain the opposite conclusions obtained by the above systematic reviews is a bias in the selection of the intervention studies included in the meta-analyses. More specifically, the systematic reviews that do not support a role of SFA in CHD (Schwingshackl and Hoffmann, 2014; Harcombe *et al.,* 2016; Hamley, 2017) excluded the Finnish Mental Hospital Study (Turpeinen *et al.,* 1979). The latter is an old study that raised many criticisms because patients were not randomized and they were assigned to each arm based on the hospital where they were recruited. Hospitals were also not allocated randomly. The FMHS was a cross-over trial, which is an inappropriate study design for the examination of mortality at long-term, since participants during the second phase may have died as a consequence of the intervention performed during the first phase (i.e., carryover effect) (Harcombe *et al.,* 2015). Finally, the control group consumed more trans fatty acids compared to the intervention group and time. The latter received the advice to increase consumption of food rich in fibre, vitamin D, or omega-3 fatty acids. These differences are likely to explain the observed differences in mortality (Heileson, 2019) and show that the FMHS study should be excluded from future systematic reviews.

Similarly to the FMSH, three other intervention studies were included in the systematic reviews that support an association between SFA intakes and CHD risk (Mozaffarian *et al.,* 2010; Hooper *et al.,* 2015; Sacks *et al.,* 2017) despite limitations in the control of confounding by trans-fatty acids, whose detrimental health effects were unknown at the time when these studies were conducted: the Oslo Diet Heart Study (ODHS) (Leren, 1970), the Medical Research Council Study (MRC) (MRC, 1968) and the Los Angeles Veteran (LA

Vet) (Dayton and Pearce, 1969) study. Even more worryingly, the most recent systematic review supporting a role of SFA in the risk of CHD (Sacks *et al.,* 2017), only included these three historical studies in addition to the FMSH study. These four biased trials also constituted more than half of the studies analysed by Mozaffarian *et al.* (2010). The latter review also inexplicably excluded two well-designed studies that would have attenuated the estimated impact of replacing SFA with PUFA (Rose et al., 1965; Ramsden et al., 2013). The above limitations corroborate the idea that the scientific evidence supporting healthy or unhealthy effects of SFA-rich products is constellated by a series of badly-designed studies and non-objective reviews.

Recent studies suggest that that the type of nutrient consumed is less important in relation to the CHD risk than the specific food matrix from which this nutrient is obtained (Michas *et al.,* 2014). This phenomenon has been defined as the "food matrix effect" and might also modulate the consequence of trans fatty acid intake on health (De Souza *et al.,* 2015). The health effects of any food product are more than the sum of the effects produced by each nutrient it contains. The specific food matrix can influence absorption, digestion, and bioavailability of all the nutrients contained in each food. Cheese contains several healthy compounds, such as short-chain fatty acids, probiotics, bioactive peptides, vitamin K-2, calcium, milk fat membrane globules. All the latter can independently affect several risk factors connected to the CHD risk, such as blood pressure, or cholesterol levels, among others (Thorning *et al.,* 2017).

Randomized intervention trials that have tested the response to dairy product consumption showed heterogeneous results. In particular, both cheese (Hjerpsted *et al.,* 2011) and non-whipped cream (Rosqvist *et al.,* 2015) have a less strong effect on total and LDL cholesterol levels compared to butter. Also, fat from dairy products has a less strong

effect on LDL cholesterol compared with fat from other sources (Soerensen *et al.,* 2014). As previously reported, the effects on blood lipids and lipoproteins do not only depend on SFA's chain length, but also on the nutrients with which SFA are replaced (Mensink, 2016). Therefore, the effects on health of dairy products are the result of complex interactions among different factors.

It appears from the above discussion that heightened complexity has evolved within individual food groups, especially for dairy products. My results indicate an increase in the mortality risk by 2% for each daily portion of nonfermented milk and butter. Although this increase in the mortality risk might seem negligible at the individual level, it has a larger impact at the population level, raising the question whether daily consumption of these food products should be discouraged in favour of a greater consumption of fermented milk which showed a neutral association with the risk of mortality.

Increased evidence is required to facilitate re-design of dietary guidelines which currently advise consumers to eat an appropriate mix from both food groups and subgroups, selecting 'nutrient-dense' options designed to increase dietary quality and promote eating patterns associated with positive health outcomes. Advice varies by age, is dependent on the outcome targeted by consumers, and negates mention of the complex array of nutrients contributed from dairy foods (including calcium, phosphorous, vitamins A, D, B2, and B12, protein, potassium, zinc, choline, magnesium, and selenium) within this advice. "Productification" adds further choices for consumers but it also adds complexity for researchers to determine the relative impacts of specific products and their fortification (particularly with vitamin D).

Finally, the cost of food influences purchase behaviour and food consumption (Kearney *et al.,* 2000), since unhealthy diets tend to be more affordable (Drewnowski, 2010) and

dietary costs represent a barrier to adopting a healthier diet among subjects with lower socio-economic status (Marmot *et al.,* 2010). As regards the Mediterranean diet, evidence of higher dietary costs associated with adherence to this dietary pattern is limited. Studies on this topic have been mostly conducted in Mediterranean countries showing a tendency towards higher costs (Lopez *et al.,* 2009; Schröder *et al.,* 2006; Vlismas *et al.,* 2010). The cost of adhering to a Mediterranean-like diet is supposed to be even higher in Nordic countries, as indicated by a Swedish short-term small-scale trial (Rydén *et al.,* 2008). However, only marginally higher dietary costs were recently found in a large UK population study, although with a greater impact on subjects with a lower socio-economic status (Tong *et al.,* 2018). Promoting adherence to the Mediterranean diet should therefore take into consideration potential consequences in terms of increased inequalities. Dietary costs could be reduced by promoting consumption of inexpensive food items among those that belong to the definition of the Mediterranean diet (i.e. vegetables, fruit, etc.).

6.2 Implications for public health

The 5th edition of the Nordic Nutritional Recommendations extensively describes the characteristics of the Mediterranean diet, its benefits, and of the scientific evidence that supports recommending it as a healthy dietary pattern (Nordic Council of Ministers, 2012). However, to recommend a regional diet such as the Mediterranean one to the rest of the world raises many questions in terms of costs, environmental sustainability, and difficulty in retrieving ingredients (Papadaki and Scott, 2002). Cultural identity can also be a barrier to the adoption of a dietary pattern that is perceived as not belonging to the norm. Therefore, it is not certain whether the Mediterranean diet can be adopted by people living in a non-Mediterranean region. The reasons for doubting generic adherence to a single

dietary pattern ideal are several. Understanding which factors can facilitate this process would increase the chances of succeeding in this task. The first problem is the perceived differences between the Mediterranean diet and the diet currently adopted by many populations. A recent qualitative study conducted in a group of middle-aged British men and women highlighted many misconceptions on the Mediterranean diet. Some of these included the belief that this diet mostly consists of cold meals (e.g., salads) instead of hot meals, or that it requires a lot of cooking skills. Perceived impact on body weight and difficulty in retrieving ingredients were other barriers to adopting the Mediterranean diet (Moore *et al.,* 2018).

The high cost of ingredients can also be a barrier to the adoption of the Mediterranean diet. A recent study performed in children and their parents, recently showed that adherence to this dietary pattern is associated with higher costs (Albuquerque *et al.,* 2017). Other adult studies came to similar conclusions, showing that a healthy diet costs more than a western-like diet (Darmon *et al.,* 2004; Lopez *et al.,* 2009; Rydén and Hagfors, 2011).

As proposed in this thesis, it is important to align nutrition advice to local cultural contexts to increase adoption by the general population. In particular, the necessity of a cultural adaptation of the Mediterranean diet to a Nordic context is one of the main factors that inspired a series of studies on a healthier version of the traditional Nordic diet. The rationale was to identify a dietary pattern including typical healthy Nordic ingredients such as root vegetables, berries, cabbages, and pears. This healthy Nordic diet (Olsen *et al.,* 2011), also known as the Baltic sea diet (Kanerva *et al.,* 2014) and the New Nordic Diet (Mithril *et al.,* 2012) consists of similar recommendations than the Mediterranean diet: large intakes of unrefined plant foods and fish. Since no large randomized controlled

intervention trials on the healthy Nordic diet exist, it is unknown whether this diet is associated with comparable health effects of the Mediterranean diet.

This study showed low adherence levels to the Mediterranean diet among European children, with the apparent exception of Swedish children. In Sweden, the Education Act foresees free school lunch provision as a mandatory part of the national food policy and dictates that school meals meet the Swedish nutrition recommendations. Free school meals are now considered a part of Swedish culture and have been offered for more than 60 years. To facilitate the adoption of healthy practices in school canteens, the Swedish National Food Agency developed guidelines for schools and youth recreation centres. These guidelines are focused on different aspects related to food and nutrition, such as sustainability, food quality and safety, taste, nutritional aspects, and meal planning. These guidelines also suggest how to integrate nutritional education into school curricula. The Karolinska Institute developed, in collaboration with the health authorities, a web-based self-assessment tool called “Skolmat Sverige” that schools use to evaluate the meals served in their canteens (Skolmat Sverige, 2020). The Swedish School Inspectorate performs regular school inspections to check for compliance with all the aspects listed in the Education Act. However, the parental FFQ used in the IDEFICS study did not cover the food consumed at school. The latter was only included in the 24HDR, although I could only analyse one single recall (Tognon *et al.,* 2014c).

Notably, the health benefits of the Mediterranean diet have often been translated into diet and nutrition guidelines and used as an inspiration for the planning of health policies, monitoring their impact on the CVD risk. This has been done by highlighting how it is possible to follow a Mediterranean-like diet by consuming foods that are typical of the Nordic diet, such as cod and salmon, local apples and berries, root vegetables, and rye,

among others. Across the WHO European region, the Nordic countries are the ones that have implemented the largest number of policies to increase the adoption of official recommendations by their citizens, which include monitoring of the "keyhole" healthy food label, among others (Renzella *et al.,* 2018). As a result, a study estimated that Swedish dietary recommendations contributed to a reduction of 32% in CVD risk for Swedish men and 27% for women (Hlebowicz *et al.,* 2013).

It is important to point out that this thesis demonstrated that a high diet quality helps prevent CVD and increase longevity, but it did not explore a potential role in treating CVD. This thesis provides evidence for CVD risk reduction in line with what previously calculated also for other chronic disease in relation to a series of dietary factors such as sodium intake, among others (National Academies of Sciences, 2019).

On the other hand, dose-response analyses (section 5.2.4) did not include a result from the VIP/MONICA cohorts, i.e. that a 32% (milk) and an 11% increased risk (butter) observed when comparing the highest (≥ 2.5 times/day) vs. the lowest (≤ 1 times/week) intake levels. The results in the H70 indicate a 20% increased mortality risk for high milk product intakes (i.e., > 400 g/day for women and 500 g/day for men) and no statistically-significant difference when comparing intermediate (2-400 g/day) with the lowest intake levels. However, these estimates might have been diluted by the fact that in the H70 cohort milk intakes were merged with yogurt and sour milk intakes whose intakes have a potentially neutral (or slightly positive) association with the risk of mortality.

6.3 Implications for the field of epidemiology

A theme connecting all the aims of this thesis was methodological improvement, aimed at creation of a more precise means to evaluate the adherence to a Mediterranean-like diet across the life course. The overarching aim was to adapt the scoring system used, reactive to the depth of detail captured within the dietary assessment methods used in this study to ensure reflection on the food culture of the populations described in this thesis and to a more accurate description of the epidemiological contextualization of a genuine Mediterranean diet. In relation to the latter, the role of typical Mediterranean and non-Mediterranean dairy products was also investigated to determine its true relation to the risk of mortality.

Dietary patterns have been studied both using a priori and a posteriori methods. The latter, which do not concern this research study, are usually based on exploratory statistical techniques such as factor and principal component analysis (Borges *et al.,* 2015). In contrast, a priori methods often consist in the use of diet scores, such as the Healthy Eating Index (Fernandes *et al.,* 2018) and the MDS that I used in this thesis. The latter, in particular, has enabled researchers to study the association between a Mediterranean-like dietary pattern and several health outcomes in many observational studies (Sofi *et al.,* 2014). Opposed to studying single food groups, the use of a score has the advantage of taking into consideration the correlation between different food groups. Diet scores represent an advancement in nutritional epidemiology since they tend to be more predictive of disease risk than single foods or nutrients thanks to their broader approach to dietary assessment (Hu, 2002). However, a certain degree of simplification is required; therefore, analyses are not sensitive to cultural influences on dietary patterns. I acknowledge that the MDS can correlate with other cultural healthy patterns since the MDS would hypothetically score as

"Mediterranean" a typical Asian diet rich in soy (a legume), rice (a cereal), pickled vegetables and fish, but low in dairy products. The MDS might have greater perceived application if considered as a tool to evaluate a universally healthy, plant-based diet. The inclusion of more typical Mediterranean foods such as wholegrain cereals and wine in the calculations of the refined MDS version, as well as the inclusion of ingredients from mixed dishes, produced stronger associations with all the outcomes I studied, highlighting the value of such amendment.

Many studies investigating the association between the MDS, chronic disease, and mortality have not made clear how mixed foods were handled during MDS calculations. I showed that adding ingredients from mixed dishes can increase the ability of the score to detect an inverse association with CVD and mortality. Olive oil is probably one of the "signature foods" of the Mediterranean diet; yet the MDS only indirectly assesses its consumption by including MUFA intakes in its calculations, when instead olive oil contains a variety of other nutrients (e.g., phenolic acids, vitamins, etc.) which concentration also depend on the olives' variety and ripening (El Riachy *et al.,* 2019). However, as discussed previously, MUFA intakes are not necessarily a marker of olive oil consumption in non-Mediterranean countries. However, to estimate oil intakes in observational studies is complicated due to the difficulty of participants to report the amount of oil for cooking or seasoning, which is uncommonly measured.

The inclusion of wine instead of total alcohol consumption in the calculations of my score also produced higher HRs. However, these results are probably explained by wine's specific consumption pattern rather than by its composition. Case-control studies have not shown any difference in the effects of wine compared to other alcoholic beverages, which were previously shown mostly in ecological studies. However, the latter are also prone to a

phenomenon known as “ecological fallacy”, which occurs when an association found at the population level cannot be confirmed at the individual level (Rimm *et al.,* 1996). Considering Mediterranean alcohol consumption patterns, I believe that wine constitutes the most “Mediterranean” of alcoholic drinks, the inclusion of moderate wine consumption into the MDS calculations is more appropriate than using moderate alcohol intakes in general. This distinction is especially important in non-Mediterranean countries where beer or other alcoholic drinks represent the major contributors to alcohol intake. Notably, the fact that wine consumption usually correlates with favourable effects on the risk of CVD is probably explained by its specific consumption pattern, rather than by its composition. Wine is more likely to be consumed in moderate amounts (and not usually associated with binge drinking) and largely at meals. This consumption pattern could explain its association with a reduced CVD risk (Augustin *et al.,* 2004; Hernandez-Hernandez *et al.,* 2015). Case-control studies have not shown any difference in the effects of wine consumption compared to other alcoholic beverages, which were previously shown mostly in ecological studies, which are prone to ecological fallacy (Rimm *et al.,* 1996).

One important question is whether my findings justify a change in the way dairy products are handled in the calculations of the MDS. It is important to note that, although high intakes of dairy product are not a typical Mediterranean habit, moderate intakes of fermented dairy products such as yogurt and cheese, are common. Also, there is no evidence that low dairy intakes reduce the risk of CVD (Guo *et al.,* 2017). Instead, the potential beneficial health effects linked to the consumption of cheese and yogurt (both typical Mediterranean foods), argues for considering these foods as typical Mediterranean options, justifying their different scoring in the MDS calculations.

In terms of addition to the existing evidence base, the findings of the research detailed here highlight the benefits of revising the MDS calculations to enable it to more accurately describe consumption of a typical Mediterranean diet. A more accurate score should include wholegrain cereals instead of total grains, olive oil consumption instead of the ratio between total unsaturated and saturated fats (e.g., one point for regular consumption), cheese and yogurt as typical Mediterranean foods, and wine instead of total alcohol consumption. Ingredients from mixed dishes should be ascertained separately during the MDS calculations. Since the study of diet is already complicated due to the absence of objective and direct measures to estimate intakes, researchers must try to avoid adding further levels of complexity. The MDS, with its endless number of different variants and associated results, makes it often impossible to compare studies, and is an example of a typical situation where agreement should be found on how to calculate the score. Although I readapted the MDS used in adults for the use in children, this was required because the IDEFICS diet dataset did not include intake quantities, but only frequencies. Previous researchers have instead created a separate index for the measurement of adherence to the Mediterranean diet: KIDMED. The KIDMED index presents some limitations, particularly since the score includes several questions that are not specifically related to a Mediterranean pattern. The latter include positively scoring the consumption of grains or dairy and avoidance of industrially-baked foods or pastries at breakfast, going to a fast-food restaurant less than once a week, or not eating sweets and candies several times per day. It is evident that, rather than assessing adherence to a Mediterranean diet score, the calculations of this score have been inflated with questions about dietary habits considered healthy to increase the likelihood of a direct correlation with health. Considering the

limitations of the latter and the already high number of existing scores, a separate score for children is therefore not necessary.

6.4 Strengths and limitations

This thesis has many strengths but, as alluded to within the sections above, also some important limitations. As for strengths, this thesis is based on cohort studies that recruited children, adults, and elderly subjects, which allowed me to answer my research questions from a life course perspective. The availability of data from large population studies, including data from long follow-ups (with the exception of the IDEFICS study), allowed me to run complex statistical models, adjusted for several potential confounders. One of my cohorts in particular, the IDEFICS study, represents one of the largest studies of children completed to date and includes data from children living in eight EU countries. The fact that each cohort used different validated dietary assessment methods allowed me to confirm that my results were consistent across different data sets and different methods used to measure diet. Finally, the results described in this thesis were proven to be robust to several sensitivity analyses.

As regards limitations, the observational nature of this study must also be considered, since it does not allow any speculations regarding potential cause-effect relationships between diet, CVD, and mortality. Although relatively straightforward for the use in epidemiological studies, the MDS also only represents a measure of relative adherence to a Mediterranean-like diet, since it is based on population-specific medians which can also be considerably lower than those observed in Mediterranean populations. Residual confounding cannot be completely ruled out, especially in those cases where only a proxy was available, as for the number of hours spent playing outdoors or in sports club (a proxy for physical activity I

used in IDEFICS) or education as a proxy for socio-economic status for example. The fact that I could not distinguish between different types of milk products in the H70 study, also limited my ability to confirm the results obtained in the VIP to cheese only. All dietary studies have the limitation that long-term dietary exposures in humans can only be indirectly assessed through interviews and questionnaires, although (in part) validated using objective markers. A limitation in the IDEFICS study was the used of Slaughter equations in children younger than 5 years despite these formulas have not been validated in this age group. However, since no valid alternative was available, I considered the use of these equations the best possible estimate of body fat mass from skinfold measurements in this age group. Finally, dietary assessment methods always carry a certain degree of error that is very difficult to estimate because of the absence of a gold standard method.

The results described in this thesis were based on different dietary assessment methods, each of which has important limitations that need to be acknowledged. Among the latter, I can mention that food records and diet histories have high respondent burden and time consuming. Since 24HDR are not suitable for children parents and caregivers were asked to report their children's diet. A consequence of this is that this type of dietary assessment can only capture dietary habits that are under parental control. Finally, FFQs are very reliant on the participants memory and are not suitable for assessing usual food intakes.

Notably, the way education was categorized in each cohort could give the impression that these categories are not comparable with each other. However, the logic we applied was the same across all thesis, i.e. to compare subjects with the highest education levels in each study with the rest of the population. In both the Swedish (VIP + Northern Sweden MONICA) and Danish (MONICA-Copenhagen) adult studies, education was categorized as university degree (> 12 years of schooling) vs. lower. The only difference was that in the

Danish MONICA study we had two additional categories for lower education (i.e., 0-7, 8-11 schooling years). Since among participants in the H70 study a university degree was very uncommon and we considered that education levels “above basics” was a reasonable way to describe the highest education levels that were achievable at that time. Finally, in the IDEFICS study education was also categorized as highly educated parents vs. lower education levels, although in this case a standardization had to be made for the sake of comparing different countries based on the International Standard Classification of Education (ISCED).

7. Conclusions

This thesis has shown that the adoption of a Mediterranean-like diet provides benefits for health across the lifespan by protecting children from obesity, adults from early death and CVD and by increasing longevity in the elderly. The sensitivity of assessments evaluating adherence to a Mediterranean-like diet can be increased. By improving the MDS I have shown that it is possible to more accurately describe the risk reduction associated to adherence to this dietary pattern, especially by adapting the score to the specific food culture of the study population and by more accurately describing the context of a Mediterranean diet. The results on intakes of dairy product show an opposite association between intake of milk and cheese in relation to the risk of total mortality, highlighting the importance of such re-evaluation, particularly where new evidence suggests a need to re-assess existing knowledge. However, this topic remains controversial since the results on cheese did not show any dose-response association with mortality in the H70 study, whereas the results on milk do not mirror the results obtained in other studies, with the exception of the Swedish study published by Michaëlsson *et al.* (2014).

Finally, from a public health perspective, adherence to a Mediterranean-like diet is low in European children although the work performed by Swedish health authorities might constitute a good model for increase adherence to the Mediterranean diet in other countries. Future studies should include large, well-designed intervention trials especially after results from the PREDIMED study were retracted from the literature.

8. Appendix 1 – Published manuscripts

The Journal of Nutrition. First published ahead of print June 27, 2012 as doi: 10.3945/jn.112.160499.

The Journal of Nutrition
Nutritional Epidemiology

The Mediterranean Diet Score and Mortality Are Inversely Associated in Adults Living in the Subarctic Region[1–3]

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Abstract

The Mediterranean diet has been widely promoted and may be associated with chronic disease prevention and a better overall health status. The aim of this study was to evaluate whether the Mediterranean diet score inversely predicted total or cause-specific mortality in a prospective population study in Northern Sweden (Västerbotten Intervention Program). The analyses were performed in 77,151 participants (whose diet was measured by means of a validated FFQ) by Cox proportional hazard models adjusted for several potential confounders. The Mediterranean diet score was inversely associated with all-cause mortality in men [HR = 0.96 (95% CI = 0.93, 0.99)] and women [HR = 0.95 (95% CI = 0.91, 0.99)], although not in obese men. In men, but not in women, the score was inversely associated with total cancer mortality [HR = 0.92 (95% CI = 0.87, 0.98)], particularly for pancreas cancer [HR = 0.82 (95% CI = 0.68, 0.99)]. Cardiovascular mortality was inversely associated with diet only in women [HR = 0.90 (95% CI = 0.82, 0.99)]. Except for alcohol [HR = 0.83 (95% CI = 0.76, 0.90)] and fruit intake [HR = 0.90 (95% CI = 0.83, 0.98)], no food item of the Mediterranean diet score independently predicted mortality. Higher scores were associated with increasing age, education, and physical activity. Moreover, healthful dietary and lifestyle-related factors additively decreased the mortality likelihood. Even in a subarctic region, increasing Mediterranean diet scores were associated with a longer life, although the protective effect of diet was of small magnitude compared with other healthful dietary and lifestyle-related factors examined. J. Nutr. doi: 10.3945/jn.112.160499.

Introduction

Numerous epidemiological studies have explored the health benefits of the Mediterranean diet and much evidence indicates that individuals who adhere to this dietary pattern are characterized by a better general health status, healthier aging, and a longer lifespan (1–5). In observational studies, the adherence to the Mediterranean diet has been found to be inversely associated with the risk of cardiovascular disease (CVD)[10] (6), certain cancers, type 2 diabetes, and also some neurodegenerative diseases (2). Furthermore, randomized controlled dietary trials have suggested that the Mediterranean diet may reduce incidence of type 2 diabetes (7), metabolic syndrome (8,9), and CVD risk factors (10,11). The favorable fatty acid profile, high fiber content, antioxidants, and phytochemicals that are typical of the Mediterranean diet as well as the synergistic interactions among its components are thought to explain some of its beneficial effects on health (1). Prospective studies have shown that following the Mediterranean diet is associated with decreased overall mortality (5,12–14) and a meta-analysis of cohort studies calculated that an increase in 2 units of the Mediterranean diet score reduced the risk of mortality by 8% (6). A Mediterranean-like diet was reported to have a beneficial effect on mortality in countries outside the Mediterranean basin (12,15,16): in Sweden, the Mediterranean dietary pattern benefits among young women have previously been reported (15).

The Mediterranean diet score was developed in the 1990s and later modified in the HALE study to also include fish intake (17). Using a refined version of this score, including PUFA, whole-grain cereals instead of total cereals, and alcohol intake,

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[2] Author disclosures: G. Tognon, L. M. Nilsson, L. Lissner, I. Johansson, G. Hallmans, B. Lindahl, and A. Winkvist, no conflicts of interest.

[3] Supplemental Tables 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[9] These authors agreed to share first authorship because of equal contribution.

[10] Abbreviations used: CVD, cardiovascular disease; FIL, food intake level; PAL, physical activity level; VIP, Västerbotten Intervention Program.

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we recently reported a protective association with mortality in elderly Swedes (18).

The aim of the present study was to evaluate whether adherence to the modified Mediterranean diet score is inversely associated with total or cause-specific mortality in the Västerbotten Intervention Program (VIP) population study in the north of Sweden, which forms one of the largest single epidemiologic cohorts for studies on nutrition and health in Europe.

Participants and Methods

Study cohort. This research was based on the VIP, which was conducted in the Västerbotten, a county situated in North Sweden with 260,000 inhabitants, 111,000 living in the main city Umeå. VIP started in the small community of Norsjö in 1985 and then spread during a couple of years to the rest of the county. During the study period, January 1, 1990 to December 31, 2008, residents of the county of Västerbotten were invited to a health survey when turning 30 (only in the period between 1990 and 1996), 40, 50, or 60 y of age. In 1996 and 2008, a group of 70-y olds was also included in the cohort. Overall, the average recruitment rate has been 59% and only limited evidence of selection bias in relation to low-income, younger age, and unemployment has been found (19). Cancer incidences demonstrate essentially no differences between the VIP cohort and the population of Västerbotten, providing evidence that it is a representative population cohort in spite of some nonparticipation (20). All participants who gave consent to participate underwent anthropometric measurements as well as measurement of blood pressure, lipid profile, and oral glucose tolerance. In addition, they completed a diet and lifestyle questionnaire, including a semiquantitative FFQ.

FFQ. During the years, different FFQ versions were used. From 1990 to 1996, the questionnaire included 84 items. Since 1997, another version has been used, including 65 items obtained after deleting some food groups (e.g., blood dishes) and merging some similar food groups (e.g., sugar and honey with marmalade and jam). Food item intakes are estimated on a fixed 9-level scale: never, a few times/y, 1–3 times/mo, 1 time/wk, 2–3 times/wk, 4–6 times/wk, 1 time/d, 2–3 times/d, and ≥4 times/d in both FFQ versions. Mealtime portion sizes were estimated with the support of 4 color pictures of a plate containing proportionally increasing amounts of main carbohydrate sources (potato/rice/pasta), main protein sources (meat/fish), and vegetables. In total, 25,864 participants completed the original, validated, 84-item VIP FFQ (21). The remaining participants completed either an older, similar, 84-item FFQ (n = 4130) or the more recent, 65-item version (n = 47,157). Macronutrient intakes were calculated from the FFQ items and portion size estimations as described elsewhere (22).

Eligibility criteria. At the end of 2008, the VIP cohort included 113,205 FFQ. With the aim of calculating the FFQ's ability to estimate plausible food intakes, food intake levels (FIL) were calculated by dividing total energy intake (calculated from reported food intakes) by the estimated basal metabolic rate (23). The physical activity level (PAL) was estimated from self-reported PAL at work and during leisure time. Briefly, physical activity at work was classified according to 4 levels (very light, light, moderate, heavy), whereas leisure physical activity was classified into 5 levels (very light, light, moderate, active, very active). For participants in the lowest level for physical activity at work, PAL varied between 1.4 and 1.9 according to increasing physical activity during leisure time. For participants characterized by the highest physical activity at work, PAL varied between 1.7 and 2.3 according to increasing physical activity during leisure time. PAL calculated in this way was previously validated by means of doubly labeled water (24).

The exclusion criteria included: participants with missing body weight (which was required to calculate FIL) or height (n = 4856) or with unrealistic FIL values (lowest 5th percentile or highest 2.5th percentile calculated by sex and FFQ version and based on the first sampling occasion for participants with repeated measures, n = 8084) as well as participants who completed <10% of food items (n = 1318) or reported an alcohol intake >50 g/d (n = 22). Remaining duplicates due to more recent sampling occasion(s) were excluded from main analyses (n = 21,391). The final study population thus encompassed 77,151 participants aged 30–60 y (including a few people aged 70 y), with a median follow-up of 10 y for all-cause mortality (2376 deaths) and 9 y for cardiovascular (680 deaths) and cancer mortality (974 deaths). Of these, 37,546 were men and 39,605 were women, 10,108 were obese (BMI ≥ 30), 20,555 had a university education, and 14,299 were smokers. Participants missing either BMI or FIL compared with the rest of the population were older, had a lower education level, were more likely to be smokers, and had a higher risk of diabetes and hypertension and were thus characterized by a higher mortality risk.

Definitions of food groups and the Mediterranean diet scores. To assess the association of diet and lifestyle factors with all-cause mortality, a refined version of the modified Mediterranean diet score (18) was calculated. This was based on existing knowledge from the scientific literature on positive health effects of whole-grain cereals (25) and moderate alcohol intake (26) as well as on the fact that PUFA and not only MUFA are the principal unsaturated fats in non-Mediterranean diets (27). The score comprised 8 components: *1*) vegetables and potatoes; *2*) fruit and juices; *3*) whole-grain cereals; *4*) fish and fish products; *5*) ratio of MUFA + PUFA to SFA; *6*) alcohol intake; *7*) meat and meat products; and *8*) dairy products. The intake of each component was adjusted to daily energy intakes of 2500 kcal (10.5 MJ) for men and 2000 kcal (8.5 MJ) for women. These levels, which have been used in other publications (17,28), were used to obtain sex-specific, energy-adjusted associations in all the analyses. In accordance with other studies based on the Mediterranean diet score, the sex and also FFQ version-specific median intakes were taken as cutoff points. A value of 1 was assigned to participants whose consumption was higher than the sex-specific median and a value of 0 to the others, with the exception of meat and dairy products, where the reverse rule was applied. The final score was obtained by summing these values, which varied from 0 (low adherence) to 8 (high adherence). An alternative index, including wine instead of total alcohol intake, was also tested. With the aim of assessing whether any single component alone of the score was able to explain the significant association with mortality, different versions of the Mediterranean diet score were created, each excluding every single component one at a time.

Ascertainment of mortality. Mortality endpoints up to 31 December 2007 were identified by linking the VIP database with the Swedish national cause-of-death registry. The 12-digit Swedish personal identification numbers were used as the linkage variable. Cardiovascular mortality was defined as the main cause of death and/or underlying cause of death, ICD-9 codes 390–438, or ICD-10 codes I00-I69 (all CVD). Cancer mortality was defined as underlying cause of death, by ICD-9 codes 140–208, or ICD-10 codes C00-C97 (all neoplasms excluding in situ and benign ones). The maximum follow-up was 18 y for total mortality and 17 y for cancer and cardiovascular mortality.

Statistical analyses. At a descriptive level, intakes per 1000 kcal of each food group included in the Mediterranean diet score and differences between men and women were calculated and tested singularly in unpaired t tests. Moreover, the determinants of high levels of the Mediterranean diet score (>4) were evaluated by means of a logistic regression, including variables such as sex, age, obesity, low levels of physical activity (PAL < 1.6), current smokers, and university education.

The association between the exposure (i.e., diet measured by means of all the versions of the Mediterranean diet score mentioned above and their single components) and mortality was tested by means of Cox proportional hazard models adjusted for age, obesity, smoking status, education, and physical activity.

Interaction between the exposure and each covariate was tested by including interaction terms in the model. Stratified estimates were calculated in case a significant interaction was found. Cause-specific analyses were also performed to test the association of the Mediterranean diet with both cancer (and subtypes such as breast, pancreas,

colorectal, stomach, prostate, and respiratory cancer) and CVD (and subtypes such as myocardial infarction and stroke).

Furthermore, the cumulative effects of an increasing number of protective, healthful, lifestyle-related factors such as never being a smoker, being physically active compared with the rest of the cohort (PAL ≥1.6), not being obese (BMI <30), or having a high Mediterranean diet score (>4) were evaluated in age-adjusted Cox proportional hazard models. In all the above-described analyses, dummy variables for missing values in physical activity and smoking variables were included in the models to retain as many participants as possible.

To exclude reverse causality bias due to participants who may have altered their diet or lifestyle in response to illness, we conducted a sensitivity analysis, adjusting for a variable describing the presence of diabetes or glucose impairment, hypertension, or pharmacological treatment for heart disease or high cholesterol at baseline. To reduce the possibility that diet or lifestyle factors may have changed in response to subclinical diseases, a second sensitivity analysis was run after excluding mortality cases during the first 2 y of follow-up. All analyses testing the association of the Mediterranean diet score with total and cause-specific mortality were repeated using a modified score considering high level of wine intake instead of total alcohol.

Finally, a sensitivity analysis aimed to confirm the main results in a subsample of participants who could be considered "adequate reporters" was performed. A ratio between FIL and PAL was calculated. Adequate reporters were defined according to 3 different definitions based on progressively more stringent criteria, i.e., exclusion of the lowest 30% (less stringent criteria), the lowest 50%, and the lowest 65% of the FIL:PAL ratio (most stringent criteria).

Statistical analyses were performed with SAS software, version 9.0 and confirmed in SPSS, version 19.0. All tests were considered significant if $P < 0.05$.

Ethics. The study protocol and data handling procedures were approved by the Regional Ethics Review Board of Northern Sweden (Dnr 07–165M). All study participants provided written informed consent and the study was conducted in accordance with the Declaration of Helsinki.

Results

Descriptive analyses. Compared with men, women had significantly higher medians of energy-adjusted intake (g/1000 kcal) for most of the food items included in the Mediterranean diet score, although men had higher medians of alcohol intakes ($P < 0.0001$) among users (**Table 1**). There were no differences between men and women in the fat ratio (MUFA + PUFA):SFA. Participants with high Mediterranean diet scores (>4) were significantly more likely to be older, more highly educated and physically active, nonsmokers and, among women, to have a BMI <30 (**Supplemental Table 1**).

TABLE 1 Energy-adjusted intake of food items included in the modified Mediterranean diet score in men and women living in a subarctic region[1]

	Men	Women
n	37,546	39,605
(MUFA + PUFA):SFA	1.19 (0.84–1.74)	1.19 (0.82–1.74)
Vegetables and potatoes, *g/d*	94.7 (29.4–218)	148 (53.6–345)*
Fruit and juices, *g/d*	49.2 (9.2–163)	109.1 (23.2–292)*
Wholegrain cereals, *g/d*	33.7 (6.5–78.2)	38.6 (8.7–87.4)*
Fish products, *g/d*	9.6 (0.5–24.7)	11.6 (0.8–29.1)*
Alcohol (among users),[2] *g/d*	2.5 (0.1–8.5)	1.5 (0.03–5.9)*
Wine (among users),[3] *g/d*	9.2 (0.3–40.0)	10.5 (0.3–43.4)*
Dairy products, *g/d*	206 (41.7–436)	219 (50.2–457)*
Meat products, *g/d*	53.0 (25.1–104)	52.0 (23.5–98.0)*

[1] Values are medians (95% CI). *Different from men, $P < 0.05$.
[2] Alcohol abstainers: *n* = 1724 (men), *n* = 3708 (women).
[3] Wine nonusers: *n* = 7917 (men), *n* = 7353 (women).

TABLE 2 Association between an increase in one level of the modified Mediterranean diet score and all-cause mortality in men and women living in a subarctic region[1]

	Cases, *n*	Men	Cases, *n*	Women
n		37,546		39,605
Model 1[2]	1453	0.94 (0.91, 0.97)***	923	0.93 (0.89, 0.97)***
Model 2[3]	1453	0.96 (0.93, 0.99)*	923	0.95 (0.91, 0.99)*
Stratified analyses[4]				
BMI <30	1225	0.95 (0.91, 0.98)**	745	0.95 (0.91, 0.99)*
BMI ≥30	228	1.03 (0.95, 1.12)	178	0.95 (0.87, 1.05)
Model 3[5]	1453	0.96 (0.93, 0.99)*	923	0.96 (0.92, 1.00)*

[1] Data are HR (95% CI). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
[2] Adjusted by age only.
[3] Adjusted by age, obesity, physical activity, smoking status, and education.
[4] *P*-interaction = 0.05 in men and nonsignificant in women.
[5] Based on the score including wine instead of alcohol intake and adjusted by age, obesity, physical activity, smoking status, and education.

Association between the Mediterranean diet score and all-cause mortality. We analyzed the association between the Mediterranean diet score and all-cause mortality separately in men and women (**Table 2**). The Mediterranean diet score was inversely associated with all-cause mortality in both men and women ($P < 0.001$), with a reduction in the strength of this association ($P < 0.05$) after covariate adjustment. The inclusion of an interaction term between the exposure and each covariate included in the model showed that obesity was an important effect modifier of the association of the Mediterranean diet score with mortality, because in both sexes, the association was significant only in participants with a BMI <30. An alternative score including wine instead of alcohol intake essentially produced the same results. Additionally, we investigated the association of the Mediterranean diet score with all-cause mortality in men and women combined and this analysis confirmed the previous results (**Supplemental Table 2**).

Comparison of the effect of diet with age, education, and lifestyle. To compare the association of diet with mortality with the association of other factors (such as age, education, and lifestyle), we ran a Cox model including all of these at the same time. Besides the expected strong effect of age class in both men [HR = 2.82 (95% CI: 2.62, 3.03)] and women [HR = 2.54 (95% CI: 2.3, 2.8)], the strongest association was observed for never smokers in both men [HR = 0.62 (95% CI: 0.56, 0.69)] and women [HR = 0.64 (95% CI: 0.56, 0. 73)] as well as for having a BMI < 30, again both in men [HR = 0.73 (95% CI: 0.63, 0.84)] and women [HR = 0.73 (95% CI: 0.62, 0.87)]. These values are stronger than those mentioned above for the Mediterranean diet score; however, the latter represent the effect of a one-step change in the Mediterranean diet score, which consists of 8 levels. The participants who did not report physical activity were characterized by an increased mortality risk, particularly among women [HR = 1.60 (95% CI: 1.36, 1.88)], suggesting that the most inactive women did not report their PAL.

Mediterranean diet score and cause-specific mortality. In a further analysis, we investigated the association between the

Mediterranean diet score and cause-specific mortality (**Table 3**). CVD mortality was inversely associated with the Mediterranean diet score in women [HR = 0.90 (95% CI: 0.82, 0.99)], although myocardial infarction [HR = 0.84 (95% CI: 0.71, 0.99)] was associated with diet while stroke was not associated with diet in men [HR = 0.98 (95% CI: 0.85, 1.13)] nor in women [HR = 1.00 (95% CI: 0.87, 1.17)], also when separately assessing the association with hemorrhagic and nonhemorrhagic stroke. Cancer mortality was inversely associated with the Mediterranean diet score in men [HR = 0.92 (95% CI: 0.87, 0.98)]. Concerning single cancer-specific mortality, the Mediterranean diet score was inversely associated with pancreas cancer mortality in men [HR = 0.82 (95% CI: 0.68, 0.99)] and tended to be inversely associated with pancreas cancer mortality also in women ($P = 0.06$), with no significant interaction with smoking status in either sex. The analyses on both total and cause-specific mortality overlapped with those obtained using a version of the Mediterranean diet score including wine instead of total alcohol intake, although the interaction with obesity disappeared. The association of the Mediterranean diet score with cause-specific mortality in men and women combined confirmed the previous results (**Supplemental Table 3**).

Relative importance of the Mediterranean diet score components. Only a few single components of the Mediterranean diet score were significantly associated with mortality (**Supplemental Table 4**). In particular, fruit was inversely associated with all-cause mortality in women [HR = 0.85 (95% CI: 0.75, 0.97)] and alcohol intake was inversely associated with mortality in men [HR = 0.84 (95% CI: 0.75, 0.93)] as well as women [HR = 0.80 (95% CI: 0.70, 0.92)]; the same was true also for wine intake. Alcohol intake was also independently inversely associated with cardiovascular mortality in both men [HR = 0.72 (95% CI: 0.60, 0.86)] and women [HR = 0.69 (95% CI: 0.50, 0.94)], but no food item was independently associated with cancer mortality. With few exceptions, the association between the Mediterranean diet score and mortality also remained significant and of a similar magnitude in the models where single components of the score were removed, one at a time, so that the total effect of the remaining components was evaluated in each such analysis (**Supplemental Table 5**).

TABLE 3 Association between the modified Mediterranean diet score and cause-specific mortality for both cancer and CVD in men and women living in a subarctic region[1]

Cause of mortality	Cases, *n*	Men	Cases, *n*	Women
n		35,950		38,034
Total cancer	493	0.92 (0.87, 0.98)**	481	0.98 (0.92, 1.03)
Breast	1	—	80	1.12 (0.97, 1.28)
Pancreas	47	0.82 (0.68, 0.99)*	45	0.83 (0.69, 1.00)
Colorectal	73	1.07 (0.93, 1.24)	54	0.91 (0.77, 1.08)
Stomach	31	1.07 (0.85, 1.34)	21	1.24 (0.95, 1.64)
Prostate	61	0.88 (0.74, 1.03)	—	—
Respiratory	68	0.86 (0.73, 1.00)	54	1.05 (0.88, 1.24)
CVD	499	0.99 (0.93, 1.04)	181	0.90 (0.82, 0.99)*
Myocardial infarction	244	0.96 (0.89, 1.04)	61	0.84 (0.71, 0.99)*
Stroke	79	0.98 (0.85, 1.13)	65	1.00 (0.87, 1.17)

[1] Data are HR (95% CI). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. CVD, cardiovascular disease.

Mediterranean diet score and other lifestyle factors. Because diet and lifestyle are strongly connected and high levels of the Mediterranean diet score were associated with smoking status, obesity, and physical activity, we evaluated the cumulative effect of diet and lifestyle factors on mortality (**Fig. 1**). Here, the age-adjusted association of a cumulative number of healthful dietary and lifestyle-related factors (i.e., high adherence to the Mediterranean diet, never smoked cigarettes, a BMI <30, and a high level of physical activity) with all-cause mortality was the strongest in the category characterized by the co-presence of all protective factors together in both men [HR = 0.38 (95% CI: 0.27, 0.54); *P*-trend < 0.0001] and women [HR = 0.38 (95% CI: 0.25, 0.57); *P*-trend < 0.0001].

Sensitivity analyses. Adjustment for a variable describing the presence of possible medical conditions at baseline that may predict diet and lifestyle changes did not affect the results on either all-cause or cause-specific mortality. Furthermore, the exclusions of early deaths (participants who died during the first 2 y of follow-up) mostly confirmed the reported results, with the exception of all-cause mortality in men, where the association with the Mediterranean diet score was no longer significant. However, the results including [HR = 0.96 (95% CI: 0.93, 0.99)] or not including early deaths [HR = 0.97 (95% CI: 0.94, 1.00)] were very similar. Moreover, the borderline significant association with pancreas cancer mortality in women ($P = 0.06$) became nonsignificant after the exclusion of early deaths ($P = 0.21$). The use of 2 alternative scores based on different energy-adjusting techniques did not materially change the results.

Finally, a sensitivity analyses based on 3 different definitions of adequate diet reporters, showed that the Mediterranean diet score was always inversely associated with both morbidity and mortality, although not all analyses were significant due to a sharp reduction of sample size, especially when the most stringent criteria were applied (>65% FIL:PAL). Respiratory cancer mortality in men and myocardial infarction in women were significantly associated with the Mediterranean diet score no matter how adequate diet reporters were defined.

Discussion

In this large, population-based study of >77,000 northern Swedes aged 30–60 y (including a few people aged 70 y), with a

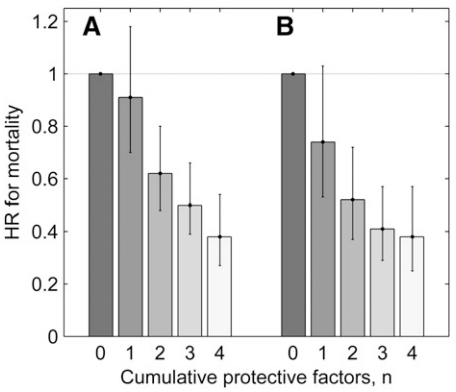

FIGURE 1 HR and 95% CI of the association between a cumulative number of protective factors related to both diet and lifestyle (including a modified Mediterranean diet score >4, being a never smoker, having a high level of physical activity, and not being obese) and total mortality (adjusted by age) in both men (*A*) and women (*B*) living in a subarctic region. *P*-trend < 0.0001 in both sexes.

maximum follow-up of 18 y (2376 deaths), the Mediterranean diet score was significantly inversely associated with all-cause mortality. In a subanalysis, it was associated with all-cause mortality only among participants with a BMI <30. In general, increasing the number of healthful dietary or lifestyle-related factors additively decreased the mortality likelihood. This dose-response to a number of lifestyle factors (not to be confused with a dose-response to number of steps within the Mediterranean diet score) suggests that it is very difficult to separate the positive effects of a healthy diet from those of a healthy lifestyle. This is an important result, because to date, not many research studies have considered the combined effects of both the Mediterranean diet and lifestyle (17,29).

The modified Mediterranean diet score is calculated based on population-specific medians and must therefore be considered a measure of relative adherence to the Mediterranean dietary pattern. The associations found in this study were robust to adjustment for several potential confounders and did not depend upon a specific component of the score. However, the magnitude of the association was not very strong (HR = 0.95 for an increase in one level of the Mediterranean diet score). Moreover, alcohol seemed to be an important component of the Mediterranean diet score, able to explain a relatively large part of the association between diet and mortality compared with other components.

The fact that the association with all-cause mortality was not found in obese participants may be explained by their different drinking habits; the interaction with obesity was not found when the Mediterranean diet score was recalculated including wine intake instead of total alcohol intake. The latter was found to be an important component of the association between diet and mortality and in this study, obese participants consumed less total alcohol compared with the nonobese participants. Because dieting is particularly common among obese participants (30), reverse causality due to changes in dietary habits during follow-up in these participants may be another plausible explanation.

We previously reported an inverse association between the Mediterranean diet score and mortality in a population study of elderly Swedes (18), although in that cohort, it was not possible to investigate the association with cause-specific mortality. The fact that in the present paper the association with CVD was confirmed only in women is a contribution to the discussion, recently started by a Canadian group, regarding the differential impact of the Mediterranean diet on cardiovascular risk in men and women (31). Some previous evidence suggested that women could respond differently to diet compared with men due in part to sex hormones (32,33). Interestingly, although a strong inverse association was found for myocardial infarction, no association was found with risk of stroke. Not many studies have investigated the association between the Mediterranean diet and stroke (34–36) and one of these had already found a null association (35). Conflicting evidence also exists regarding the role of hypercholesterolemia in ischemic stroke development (37) and this might be the main reason for the contradictory results. Additionally, stroke is a heterogeneous disease that involves both small and large vessels and this may have contributed to the lack of association we observed with the Mediterranean diet score. On the contrary, myocardial infarction is more homogeneous and primarily atherosclerotic, which may be one reason for the strong association with diet.

With regard to cancer, epidemiological studies have been investigating the protective role of the Mediterranean diet, with most research conducted within the last 5–6 y. A recent review of observational studies found 12 studies (7 cohort and 5 case-control) of which 10 (6 cohorts and 4 case-control) provided some evidence that the Mediterranean diet was associated with a reduced risk of both cancer incidence and mortality (38). In our study, the latter was inversely associated with the Mediterranean diet score only in men, with an inverse association for pancreatic cancer mortality, only to a limited extent confirmed by a borderline result in women ($P = 0.06$). Pancreatic cancer is one of the most aggressive among neoplasms as well as a very difficult disease to detect and treat, making prevention one of the main strategies to reduce mortality (39). A few risk factors have been implicated in the etiology of this malady, with cigarette smoking being the most consistent and accounting for ~20% of incident cases (40). Our results thus help clarify the questioned relationship between diet and pancreatic cancer morbidity and mortality. To the best of our knowledge, this is the first study showing an inverse association with mortality for pancreatic cancer, notably without a significant interaction with smoking status. The inverse association of fruit and vegetable intake with the risk of this neoplasm is indeed still debated (41), but it is important to note that, though a high fruit intake was not associated with cancer mortality in general, in this study it was strongly inversely associated with pancreatic cancer mortality in women (data not shown). However, some years ago, the World Cancer Research Fund (WCRF) reported a convincing inverse association with folate-rich food intakes (42). An inverse association of a healthy lifestyle score with pancreatic cancer incidence was recently found in the NIH-AARP cohort study (43).

This study has both strengths and limitations, the former being the large size, which allowed us to study the association of the Mediterranean diet score with all-cause mortality as well as cancer-specific mortality both stratified by sex and in the whole population. Other strengths include the use of a validated FFQ and the possibility of adjusting the statistical analyses for several different confounders. A limitation is that participants who lacked information on either anthropometry or FIL were excluded in the analyses of the association between diet and mortality. Because they had a higher mortality rate, their exclusion in our analyses may have distorted our results. Another main limitation is the fact that we cannot fully exclude potential reverse causation due to diet changes during follow-up. However, we performed a sensitivity analysis excluding early deaths, because participants with CVD or cancer or with early symptoms of these diseases may have altered their diet or lifestyle as a consequence. Notably, adjusting for the presence of diabetes, impaired glucose tolerance, hypertension, or for taking medications related to heart disease or high cholesterol did not materially change the results on either all-cause or cause-specific mortality.

In conclusion, relative adherence to a Mediterranean diet pattern is associated with a longer life and a reduction in cardiovascular mortality as well as a suggestive reduction of cancer mortality, particularly in relation to pancreatic cancer, although with a limited magnitude. Alcohol seemed to be an important component of this association, particularly in relation to the cardiovascular mortality. High scores for this dietary pattern are more frequent in participants with a generally healthy lifestyle and indeed, modifiable risk factors such as weight, smoking status, physical activity, and diet are associated with a lower mortality risk, particularly in relation to CVD fatalities. However, an important take-home message is that once again it is clear that the concept behind the Mediterranean diet is as nonspecific as the Mediterranean Sea population, which is composed of people living in more than 20 countries, each with its own culinary identity (44). Although the single effect of a

healthy diet cannot be fully extrapolated from a healthy life overall, the benefits of the Mediterranean dietary pattern go beyond the geographical area where it was first described (4) and can thus be borrowed by other populations, even by those living much further away.

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ONLINE SUPPORTING MATERIAL[1]

[1] Due to a deviation of sex variable from the proportional hazard assumptions as confirmed by Schoenfeld's test ($P = 0.03$), analyses on the overall population were performed either by including the STRATA option in the SAS program for stratified estimate or by including interaction terms between sex and follow up time. Both corrections gave exactly the same results as the simple adjustment by sex, as expected by the fact that men and women curves in the log-log survival plot were nearly parallel (data not shown).

SUPPLEMENTAL TABLE 1 Determinants of high adherence level to the Mediterranean diet (Mediterranean diet score > 4), evaluated by logistic regression in men and women living in a sub-artic region[1,2].

	Men, n = 37,546	Women, n = 39,605
Age strata (10 y)	1.31 (1.28, 1.34)***	1.58 (1.54, 1.62)***
BMI ≥ 30	1.00 (0.94, 1.07)	0.92 (0.86, 0.98)**
Low physical activity[3]	0.80 (0.77, 0.84)***	0.72 (0.70, 0.76)***
University education	1.73 (1.65, 1.82)***	1.52 (1.45, 1.59)***
Current smokers	0.75 (0.71, 0.80)***	0.81 (0.77, 0.85)***

[1]Data are expressed as Odds Ratios (95% CI). [2]All variables were included in the same model and thus adjusted for all the other covariates. [3]Defined as Physical Activity Level (PAL) < 1.6. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

SUPPLEMENTAL TABLE 2 Association between an increase in one level of the Mediterranean diet score and mortality in men and women living in a sub-artic region[1].

	Cases, *n*	Men and Women, *n* = 77,151
Model 1[2]	2,376	0.93 (0.90, 0.95)***
Model 2[3]	2,376	0.95 (0.93, 0.98)***
Stratified analyses[4]		
BMI < 30	1,970	0.95 (0.92, 0.97)***
BMI ≥ 30	406	0.99 (0.93, 1.06)
Model 3[5]	2,376	0.96 (0.93, 0.98)***

[1]Data are expressed as Hazard Ratios (95% CI). [2]Only age adjusted. [2]Adjusted by age, obesity, physical activity, smoking status and education. [3]*P* value for interaction: 0.05 in men, non-significant in women. [4]Based on the score including wine instead of alcohol intake and adjusted for age, obesity, physical activity, smoking status and education. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

SUPPLEMENTAL TABLE 3 Association between the Mediterranean diet score and cause-specific mortality for both cancer and cardiovascular diseases in men and women living in a sub-artic region[1].

Cause of mortality	Cases, *n*	Men and Women, *n* = 73,984
Total cancer	974	0.95 (0.91, 0.98)**
Breast	80	-
Pancreas	92	0.82 (0.72, 0.94)**
Colorectal	127	0.99 (0.89, 1.11)
Stomach	52	1.14 (0.96, 1.35)
Prostate	61	-
Respiratory	122	0.93 (0.83, 1.04)
Cardiovascular diseases	680	0.96 (0.92, 1.01)
Myocardial infarction	305	0.94 (0.87, 1.01)
Stroke	144	0.99 (0.89, 1.09)

[1]Data are expressed as Hazard Ratios (95% CI). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

SUPPLEMENTAL TABLE 4 Association between high/low levels of single components of the modified Mediterranean diet score and CVD[1] mortality in men and women living in a sub-artic region[2].

	All-cause mortality		Cardiovascular mortality		Cancer mortality	
	Men, *n* = 37,546	Women, *n* = 39,605	Men, *n* = 35,950	Women, *n* = 38,034	Men, *n* = 35,950	Women, *n* = 38,034
High intake of:						
- (MUFA + PUFA)/SFA	1.00 (0.90, 1.12)	1.04 (0.91, 1.18)	1.12 (0.93, 1.36)	0.94 (0.70, 1.26)	0.94 (0.77, 1.14)	1.09 (0.91, 1.31)
- Vegetables and potatoes	0.93 (0.84, 1.03)	0.96 (0.84, 1.09)	0.93 (0.78, 1.12)	0.92 (0.68, 1.23)	0.88 (0.73, 1.05)	0.88 (0.74, 1.06)
- Fruit and juices	0.93 (0.84, 1.03)	0.85 (0.75, 0.97)*	0.95 (0.79, 1.14)	0.75 (0.56, 1.02)	0.84 (0.70, 1.00)	0.89 (0.74, 1.07)
- Wholegrain cereals	0.93 (0.84, 1.03)	1.03 (0.90, 1.17)	1.14 (0.95, 1.36)	1.04 (0.78, 1.40)	1.00 (0.84, 1.20)	1.02 (0.85, 1.22)
- Fish products	1.05 (0.95, 1.16)	1.02 (0.89, 1.16)	1.10 (0.93, 1.32)	1.03 (0.77, 1.39)	0.85 (0.71, 1.01)	0.97 (0.81, 1.17)
- Alcohol	0.84 (0.75, 0.93)**	0.80 (0.70, 0.92)**	0.72 (0.60, 0.86)*	0.69 (0.50, 0.94)***	0.94 (0.78, 1.12)	0.85 (0.71, 1.02)
- Wine	0.82 (0.73, 0.91)***	0.84 (0.74, 0.96)*	0.68 (0.56, 0.82)***	0.78 (0.58, 1.06)	1.03 (0.86, 1.24)	0.90 (0.75, 1.08)
Low intake of:						
- Dairy products	0.96 (0.87, 1.07)	0.93 (0.81, 1.06)	1.00 (0.84, 1.20)	0.90 (0.67, 1.21)	1.04 (0.87, 1.24)	0.93 (0.78, 1.12)
- Meat products	0.97 (0.87, 1.08)	0.91 (0.80, 1.03)	0.97 (0.81, 1.17)	0.82 (0.61, 1.10)	1.01 (0.84, 1.22)	0.99 (0.82, 1.18)

[1]Cardiovascular diseases. [2]Data are expressed as Hazard Ratios (95% CI). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

SUPPLEMENTAL TABLE 5 Association between different versions of the modified Mediterranean diet score recalculated with the exclusion of one of its components and cardiovascular mortality in men and women separately and in both genders combined in men and women living in a sub-artic region[1].

	All-cause mortality		Cardiovascular mortality		Cancer mortality	
	Men, *n* = 37,546	Women, *n* = 39,605	Men, *n* = 35,950	Women, *n* = 38,034	Men, *n* = 35,950	Women, *n* = 38,034
Fat ratio	0.95 (0.92, 0.98)**	0.93 (0.89, 0.98)**	0.97 (0.91, 1.03)	0.89 (0.80, 0.98)*	0.91 (0.86, 0.97)**	0.96 (0.90, 1.02)
Vegetables and potatoes	0.96 (0.92, 0.99)*	0.95 (0.91, 0.99)*	0.97 (0.91, 1.03)	0.89 (0.80, 0.99)*	0.93 (0.87, 0.99)*	0.97 (0.91, 1.04)
Fruit	0.96 (0.93, 0.99)*	0.96 (0.92, 1.00)*	0.99 (0.93, 1.05)	0.92 (0.83, 1.01)	0.93 (0.87, 0.99)*	0.98 (0.93, 1.05)
Wholegrain cereals	0.96 (0.93, 0.99)*	0.94 (0.90, 0.98)*	0.99 (0.93, 1.05)	0.89 (0.80, 0.98)*	0.93 (0.87, 0.99)*	0.96 (0.91, 1.02)
Fish	0.94 (0.91, 0.98)*	0.94 (0.90, 0.99)**	0.97 (0.91, 1.03)	0.89 (0.80, 0.98)*	0.93 (0.87, 0.99)*	0.98 (0.92, 1.04)
Alcohol	0.97 (0.94, 1.01)	0.97 (0.93, 1.01)	1.02 (0.96, 1.09)	0.93 (0.84, 1.02)	0.92 (0.86, 0.98)**	0.99 (0.93, 1.05)
Dairy products	0.95 (0.92, 0.99)*	0.95 (0.91, 0.99)*	0.98 (0.92, 1.05)	0.90 (0.81, 0.99)*	0.90 (0.85, 0.96)**	0.98 (0.92, 1.04)
Meat products	0.96 (0.93, 0.99)*	0.96 (0.92, 1.00)	0.99 (0.93, 1.05)	0.92 (0.84, 1.01)	0.92 (0.87, 0.98)**	0.98 (0.92, 1.03)

[1]Data are expressed as Hazard Ratios (95% CI). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

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The Mediterranean diet in relation to mortality and CVD: a Danish cohort study

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Abstract

The aim of the present study was to determine whether the Mediterranean Diet Score (MDS) is associated with reduced total mortality, cardiovascular incidence and mortality in a Danish population. Analyses were performed on 1849 men and women sampled during the 1982–83 Danish MONICA (MONItoring trends and determinants of Cardiovascular disease) population study, whose diet was assessed by means of a validated 7 d food record. The adherence to a Mediterranean dietary pattern was calculated by three different scores: one based on a classification excluding ingredients from mixed dishes and recipes (score 1); another based on a classification including ingredients (score 2); the last one based on a variant of the latter including wine instead of alcohol intake (score 3). The association between these scores and, respectively, total mortality, cardiovascular incidence and mortality was tested by a Cox proportional hazards model adjusted for several potential confounders of the association. Generally, all three scores were inversely associated with the end-points, although associations with score 1 did not reach statistical significance. Score 2 was inversely associated with total mortality (hazard ratio 0·94; 95 % CI 0·88, 0·99). This association was confirmed for total cardiovascular as well as myocardial infarction (MI) incidence and mortality, but not for stroke. Score 3 was slightly more associated with the same outcomes. All associations were also resistant to adjustment for covariates related to potential CVD pathways, such as blood lipids, blood pressure and weight change after 11 years of follow-up. In a Danish cohort, the MDS was inversely associated with total mortality and with cardiovascular and MI incidence and mortality, but not with stroke incidence or mortality.

Key words: Mediterranean diet: Mortality: Cardiovascular incidence: Mortality

Lifestyle and eating habits are key modifiable factors that people can change to decrease chronic disease risk. Poor diet, in particular, has been convincingly linked to the incidence of CVD and some forms of cancer[1,2], which are responsible for most of the disease burdens in the majority of developed countries[3].

Dietary recommendations promoting healthy dietary patterns including whole-grain foods, legumes, vegetables and fruit, and suggesting that the intake of full-fat dairy products, sweets and red meat be limited, are an emerging approach for the prevention of several diseases, and the Mediterranean-style diet embodies many of these recommendations[4]. Indeed, in a recent meta-analysis, the Mediterranean eating pattern has turned out to be more effective than low-fat diets in inducing important long-term changes in cardiovascular risk factors and inflammatory markers[5].

Greater adherence to a Mediterranean-like dietary pattern, in particular, has been shown to be associated with improvement in health status[6,7] as well as inversely associated with both premature[8] and total mortality[9,10]. The latter effect has been observed in countries both inside[11] and outside[12–14] the Mediterranean basin, for instance, in Nordic countries[12], where we recently reported an inverse association with mortality both in elderly[15,16] and in middle-aged adults living in Sweden[17].

Dietary patterns in the Mediterranean countries are changing rapidly, with an increased consumption of animal products and saturated fats and a decline in the intake of vegetable-based foodstuffs[18–20]. On the other hand,

Abbreviations: FIL, food intake level; HR, hazard ratio; MDS, Mediterranean Diet Score; MI, myocardial infarction; PAL, physical activity level.

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Nordic populations have paradoxically increased their intakes of typical Mediterranean items such as fruit, vegetables and wine between 1970 and mid-2000[21–23].

The aim of the present study was to determine whether the Mediterranean Diet Score (MDS) was inversely associated with all-cause mortality as well as cardiovascular incidence and mortality in the Danish MONICA (MONItoring trends and determinants of Cardiovascular disease) population study.

Subjects and methods

Subjects and measures

In 1982, a sample of 4807 subjects was randomly selected from the Central Person Register of citizens born in 1922, 1932, 1942 and 1952 living in eleven municipalities in the Copenhagen County[24]. Of these, 226 were of non-Danish origin. Among the 4581 invited, 3608 participated in a subsequent health examination in 1982–3[25]. The non-respondents have been described in detail elsewhere[26]. The present study was part of the Danish MONICA project, an international study conducted under the auspices of the WHO to monitor CVD trends and determinants. One subject was excluded due to missing BMI information at baseline and three subjects for missing blood test results. The cohort described here included 1849 subjects (948 women) with complete information on diet intake and anthropometry and 1348 subjects (701 women) who also had results for blood tests and blood pressure.

The participants filled in a questionnaire describing their highest grade of education or highest year of regular schooling classified into 0–7, 8–11 and ≥12 years, their cigarette smoking habits and their leisure time activity. The latter was categorised into four classes: inactive; light (<4 h/week); moderate (>4 h/week); energetic (>4 h/week).

Body weight was measured to the nearest 0·1 kg using a lever balance, with subjects dressed in light clothing or underwear. Height was measured without shoes to the nearest 1 cm. Weight change information after 11 years of follow-up was available for a subsample of 1348 subjects.

BMR (kcal/d) was calculated according to Schofield equations for subjects aged 30–59 years[27]:

$$\text{BMR} = 11{\cdot}5\,\text{weight (kg)} + 873\,\text{(men)}.$$

$$\text{BMR} = 8{\cdot}3\,\text{weight (kg)} + 846\,\text{(women)}.$$

Food intake level (FIL) was then calculated based on the ratio of reported energy intake to BMR. Both systolic and diastolic blood pressures were measured with a London School of Hygiene sphygmomanometer, using one of the three different cuffs. Duplicate measurements were taken on the left arm after a minimum of 5 min of rest in a supine position. Means of duplicate measures were calculated and used in the analyses described below. Blood samples were drawn after a 12 h overnight fast. A commercial enzymatic method was used (Boehringer Mannheim) to measure the concentrations of total cholesterol, HDL-cholesterol and TAG (mmol/l) in the serum[28]. The total cholesterol:HDL-cholesterol ratio was calculated, and a value greater than 4 was arbitrarily chosen as a threshold of a risky lipid profile.

Dietary assessment

Information on diet was obtained by means of a four-page (A3 format) weighed 7 d food record. The front page contained instructions for correct completion of the diet records together with the average household weights of nineteen frequently consumed foods, e.g. the white of an egg and a slice of bread. For butter and liver pâté, three examples were given of typical amounts put on a slice of bread. On the next pages, 100 foods were listed in the following nine subgroups: dairy products; bread and cereal grains; fats; cold cuts, etc.; vegetables; fruit; meat (including poultry and fish); drinks; miscellaneous items. The last page included space to record foods that did not fit into any of the nine groups, such as mixed dishes, home-made salads and cakes.

The participants were also given thorough verbal instructions on how to fill out the food record. It was emphasised that the diet record be completed during a typical 7 d period including a minimum of non-habitual social activities. Food quantities were expressed in grams, estimated as accurately as possible or preferably weighed. The same trained dietitian manually checked and coded all food records into dietary components.

Definitions of food groups and of the Mediterranean Diet Score

To assess the association of diet and lifestyle factors with all-cause mortality, a version of the modified MDS[29] adapted to the MONICA diet questionnaire was calculated. The score comprised eight positive or negative components: (1) ratio of (monounsaturated + polyunsaturated):saturated fats; (2) vegetables; (3) fruit; (4) cereal grains; (5) fish and fish products; (6) alcohol intake; (7) meat, meat products and eggs (negative); (8) dairy products (negative). As has been described previously[9,29], the intake of each component was adjusted to the daily energy intakes of 10·467 kJ (2500 kcal) for men and 8·374 kJ (2000 kcal) for women, in order to obtain energy-adjusted associations in all the analyses. As sex-specific median intakes were taken as cut-off points, the final score must be considered as a measure of relative adherence to the Mediterranean dietary pattern. A value of 1 was assigned to subjects whose consumption was higher than the sex-specific median and 0 to the others, with the exception of meat and dairy products, where the reverse rule was applied. The final score was obtained by summing these values, and it varied from 0 (low adherence) to 8 (high adherence). Food groups were defined based on two different procedures, from which two different scores were produced. The first procedure included the identification of all the possible food items that could be classified according to the above-mentioned food categories (vegetables, fruit, etc.), but excluding mixed dishes (score 1). The second procedure included food items classified into food groups as in score 1, but now including ingredients extrapolated from mixed dishes or recipes (score 2). Finally, a variant of score 2 was created, now including wine instead of total alcohol intake (score 3).

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Endpoints

Information on all-cause mortality as well as on CVD or myocardial infarction (MI) incidence and mortality was retrieved from the National Patient Registry of Hospital Discharges, the Cause of Death Register and the Central Person Register. The subjects were followed until 8 July 2007 for an average of 14 years. Survival time was parameterised in terms of lifetime until death or censoring event in years, with left truncation at the age of entrance into the study being equal to baseline examination. Total CVD cases were identified based on codes 390–458 (ICD8) and I00–I99 (ICD10) and MI cases were identified based on codes 410 (ICD8) and I21 (ICD10), while stroke cases were identified based on codes 430–434 + 436 (ICD8) and I60–I64 (ICD10). Analyses on CVD, MI and stroke incidence (both fatal and non-fatal cases) and mortality were performed given that there was no CVD, MI or stroke at baseline.

Statistical analyses

The associations of the three above-mentioned diet scores with mortality were determined by means of Cox proportional hazards models, either adjusted only by age or adjusted for potential confounders such as sex, age, obesity, smoking status (two dummy variables were included, describing pack years greater than 0 but less than 30 and pack years greater than 30, keeping the 'never smoked' category as the reference), education (the lowest level *v.* higher levels) and physical activity (see the above-mentioned categories). The latter model was then considered the main model. Additionally, in order to determine possible pathways of effects, we performed some confirmatory analyses adjusting for blood pressure (systolic and diastolic, included as continuous variables), blood lipids (TAG, included as a continuous variable, and total cholesterol:HDL-cholesterol ratio >4) and weight change (including two dummy variables describing weight change >3 kg or <-3 kg and keeping a weight change of 0 to ± 3 kg as the reference category). All-cause mortality as well as cause-specific incidence and mortality, i.e. total CVD, MI and stroke, were studied in association with the MDS.

Effect modification of exposure (MDS) by each covariate was tested in the main model by including interaction terms. Stratified estimates were calculated in case a significant interaction was found. The association of single components of the MDS was also tested separately, by including as exposure a variable describing an intake over the sex-specific medians (fat ratio, vegetables, fruit, cereal grains, fish and alcohol) or below the median (meat and dairy products). To reduce bias for changes in diet or lifestyle factors due to subclinical disease, the main model was also run after excluding the first 2 years of follow-up. In analyses, different versions of the MDS were included, and these were excluded one at a time, and also single components of these scores were tested (while keeping all other components) in order to determine whether any associations could be explained by a single component.

With the aim of determining the additive effects of an increasing number of risk factors such as ever been a smoker, having a low physical activity level (PAL) (<4 h/week) and obesity (BMI ≥ 30 kg/m^2) and not consuming a Mediterranean-like diet (MDS ≤ 4), a new score was calculated, assigning an additional point for each of the above-mentioned risk factors. Different hazard ratios (HR) and 95% CI describing the association (adjusted by sex) of each level of the score with all-cause mortality were calculated and depicted in a histogram.

A sensitivity analysis was performed, aiming to confirm the main results in a subsample of subjects who could be considered 'adequate reporters'. The ratio of FIL:PAL was calculated. Adequate reporters were defined according to three different definitions based on increasingly stringent criteria, i.e. exclusion of the lowest 30% (less stringent criterion) and of the lowest 50% and of the lowest 65% of the FIL:PAL ratio (most stringent criteria).

Statistical analyses were performed with SAS software (version 9.0; SAS Institute). All tests were considered significant if $P<0{\cdot}05$.

Bioethics

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki (1989) of the World Medical Association, and all procedures involving human subjects were approved by the Ethics Committee of the Medicine Faculty of the Copenhagen County. All subjects were informed of the aims and procedures of the study, and they gave their written consent.

Results

Cohort description

The population consisted of 948 women (51·3%) and 901 men (48·7%). Only a small number were obese (*n* 138, 7·5%).

Table 1. Intake value of each food group included in the Mediterranean Diet Score, adjusted to 8·374 MJ (2000 kcal) in women and 10·467 MJ (2500 kcal) in men*

(Medians with their 5th–95th percentiles)

	Median	5th–95th percentile
(Monounsaturated + polyunsaturated): saturated fats	1·1	0·8–1·6
Vegetables (g/d)	192·1	79·0–418·5
Fruit (g/d)	109·8	0–401·5
Cereal grains (g/d)	179·9	94·3–297·0
Fish and shellfish (g/d)	25·2	0–87·5
Alcohol among users (g/d)	15·5	2·0–62·6
Wine among users (g/d)	2·5	0·8–13·0
Dairy products (g/d)	297·4	55·0–750·6
Meat products (g/d)	182·3	95·6–310·5
Energy intake (kJ/d) in men	10 571·7	6514·7–15 993·6
Energy intake (kJ/d) in women	7460·9	4450·6–11 852·8
FIL (energy intake:BMR)	1·4	0·8–2·1
Mediterranean Diet Score 1	5	2–7
Mediterranean Diet Score 2	4	2–6
Mediterranean Diet Score 3	4	2–6

FIL, food intake level.
* Energy intake and the ratio of energy intake:BMR are also reported.

Table 2. Survival analyses on the association between an increase in one unit of the three different versions of the Mediterranean Diet Score (scores 1, 2 and 3) and all-cause mortality as well as cardiovascular mortality and incidence

(Hazard ratios (HR) and 95 % confidence intervals)

		All-cause mortality (*n* 553)		CVD				Myocardial infarction				Stroke			
				Incidence† (*n* 755)		Mortality (*n* 223)		Incidence† (*n* 161)		Mortality (*n* 64)		Incidence† (*n* 167)		Mortality (*n* 40)	
	n	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI
Score 1 (main model)‡	1849	0·95	0·91, 1·00	0·97	0·93, 1·02	0·96	0·89, 1·05	1·00	0·91, 1·01	0·88	0·76, 1·02	0·96	0·88, 1·06	1·06	0·88, 1·29
Age-only-adjusted model	1849	0·97	0·92, 1·02	0·97	0·93, 1·02	0·98	0·91, 1·06	0·99	0·90, 1·08	0·90	0·78, 1·05	0·96	0·87, 1·06	1·06	0·88, 1·29
Score 2 (main model)‡	1849	0·94*	0·88, 0·99	0·94*	0·89, 0·99	0·90*	0·82, 0·99	0·89*	0·80, 1·00	0·80*	0·67, 0·96	0·96	0·86, 1·07	1·03	0·82, 1·30
Age-only-adjusted model	1849	0·95	0·89, 1·00	0·94*	0·89, 0·99	0·92	0·84, 1·00	0·91	0·82, 1·02	0·84*	0·70, 0·99	0·95	0·86, 1·06	1·01	0·81, 1·25
Score 3 (main model)‡	1849	0·93*	0·87, 0·98	0·92***	0·87, 0·97	0·87**	0·79, 0·95	0·89*	0·80, 1·00	0·79**	0·66, 0·94	0·93	0·84, 1·04	0·97	0·77, 1·21
Age-only-adjusted model	1849	0·93*	0·88, 0·99	0·91***	0·87, 0·96	0·88**	0·80, 0·96	0·91	0·82, 1·01	0·80**	0·67, 0·95	0·92	0·83, 1·02	0·94	0·76, 1·17

Values were significantly different: * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$.

† Fatal and non-fatal cases.

‡ Adjusted for sex, BMI, education, physical activity and cigarette smoking.

A total of 476 (25·7 %) had never smoked cigarettes, while 303 (16·4 %) smoked more than thirty pack years. Finally, 241 subjects (13 %) abstained from any alcohol intake. Among the cardiovascular risk factors, 450 subjects (24·3 %) had high blood pressure (either systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg), almost half of the population had either a total cholesterol:HDL-cholesterol ratio > 4 (*n* 955, 51·6 %), while about a third had gained more than 3 kg at follow-up (*n* 666, 36·0 %).

Energy-adjusted median values of each score component (Table 1) showed that the ratio of unsaturated:saturated fats was 1·1 (5th–95th percentiles: 0·8–1·6), indicating a similar level of consumption of each type of fat. Vegetable intake was higher than fruit intake, while the median intake for dairy products was almost 300 g/d. Median alcohol intake among users was about one standard unit and a half (15·5 g/d), while median wine intake was 2·5 g/d. The median reported daily energy intakes were 10 571·7 MJ (2525 kcal) in men and 7460·9 MJ (1782 kcal) in women. The ratio of energy intake:BMR was 1·4 (5th–95th percentiles: 0·8–2·1), indicating a good estimation of energy intake compared with the metabolic rate. The differences among the median values of the three scores were very small.

Main analyses and effect modification

The association between the three different versions of the MDS (scores 1, 2 and 3) and all-cause mortality as well as CVD incidence and mortality was assessed (Table 2), adjusting for only age or for different potential confounders such as sex, age, BMI, education, physical activity and cigarette smoking (main model). Since no statistical interaction ($P > 0{\cdot}05$) with sex was found in relation to almost all outcomes, all analyses were conducted on men and women together, adjusting for sex.

Results were, in general, in the direction of an inverse association between the three scores and all the outcomes, with the exception that the association between the MDS calculated excluding mixed dishes and recipes (score 1) did not reach statistical significance in either the main model or in the age-only-adjusted model. In contrast, the second version of the MDS, including the ingredients from mixed dishes and recipes (score 2), was inversely associated with all outcomes except with stroke incidence (HR 0·96; 95 % CI 0·86, 1·07) and mortality (HR 1·03; 95 % CI 0·82, 1·30). In the age-only-adjusted model, score 2 was significantly associated only with CVD incidence and MI mortality. Finally, score 2, recalculated to include wine instead of total alcohol intake (score 3), was significantly associated with all outcomes, but, again, not with stroke.

No effect modification was found when testing the interaction with potential confounders, with the exception of the association between the MDS and MI mortality, which was significant only at low PAL (moderate activity < 4 h/week) (score 3: HR 0·61; 95 % CI 0·43, 0·86; P for interaction: 0·04).

In order to determine possible pathways of effects in relation to the cardiovascular risk, the main model was further adjusted for blood lipids and blood pressure as well as for

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the latter plus weight change (Table 3). With the exception of MI incidence, which did not resist the adjustment for weight change (HR 0·91; 95% CI 0·79, 1·05), the results obtained after these additional adjustments were in line with the previous ones (main model, Table 2) or slightly stronger. Finally, the exclusion of early deaths (i.e. subjects who died during the first 2 years of follow-up) did not materially change the results, with the exception, again, of MI incidence, where association with the score was only of borderline significance (HR 0·91; 95% CI 0·81, 1·02).

Supplementary analyses

Table 4 summarises the results of a supplementary analysis, where the association of all endpoints with different versions of score 3 was determined. The alternative versions were created by excluding, one at a time, each single score component to check if any of these could explain alone the association of the score. The results show that the association was robust, and in the few cases where the association was not significant, a borderline association was found. The association of single MDS components (high or low intake according to median values) with all the endpoints investigated revealed only a few significant results (Table 5). The most robust one was found with high fruit intake, which was inversely associated with all-cause mortality (HR 0·74; 95% CI 0·62, 0·88), CVD incidence (HR 0·86; 95% CI 0·75, 1·00) and mortality (HR 0·72; 95% CI 0·55, 0·94). Also, vegetables showed some significant associations with all-cause mortality (HR 0·81; 95% CI 0·68, 0·96) as well as with MI incidence (HR 0·73; 95% CI 0·54, 1·00) and mortality (HR 0·58; 95% CI 0·35, 0·96).

The results of the sensitivity analyses, based on three different definitions of adequate diet reporters (>30% FIL:PAL, >50% and >65%), showed that although score 3 was always inversely associated with both incidence and mortality, not all these analyses were statistically significant due to a highly reduced number of cases, especially when the most stringent criterion was applied (>65% FIL:PAL). No large variations of these estimates were observed comparing these three analyses. Both all-cause mortality and CVD mortality were shown to be the strongest associations, since they were statistically significant no matter how adequate diet reporters were defined.

Mediterranean Diet Score and other lifestyle factors

Diet and lifestyle are strongly associated, and the level of the MDS was associated with smoking status, obesity and physical activity. We determined the cumulative effect of diet and lifestyle-related risk factors on mortality (not to be confused with a dose response to the number of levels of the MDS) by means of an additional score (Fig. 1). With increased number of risk factors (i.e. ever been a smoker, having a low physical activity level, being obese and not consuming a Mediterranean-like diet) the mortality risk increased linearly. In the highest risk category (three or four risk factors) the sex-adjusted association will all-cause

Table 3. Survival analyses on the association between an increase in one unit of Mediterranean Diet Score 3 and all-cause mortality as well as cardiovascular (CV) mortality and incidence (Hazard ratios (HR) and 95% confidence intervals)

		All-cause mortality (*n* 553)		CVD				Myocardial infarction				Stroke			
				Incidence† (*n* 755)		Mortality (*n* 223)		Incidence† (*n* 161)		Mortality (*n* 64)		Incidence† (*n* 167)		Mortality (*n* 40)	
	n	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Covariates related to CV risk‡§	1849	0·93**	0·87, 0·98	0·93**	0·88, 0·97	0·86**	0·79, 0·95	0·89*	0·80, 1·00	0·79**	0·66, 0·94	0·93	0·84, 1·04	0·97	0·78, 1·22
Weight change‡‖	1348	0·90*	0·83, 0·98	0·91**	0·85, 0·97	0·81**	0·71, 0·93	0·91	0·79, 1·05	0·73*	0·56, 0·96	0·89	0·78, 1·02	0·98	0·72, 1·32
Excluding early deaths‡	1822	0·93*	0·88, 0·99	0·90***	0·85, 0·95	0·84***	0·77, 0·93	0·91	0·81, 1·02	0·80*	0·67, 0·96	0·93	0·84, 1·04	0·96	0·76, 1·20

Values were significantly different: * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$.
† Fatal and non-fatal cases.
‡ Adjusted for sex, BMI, education, physical activity and cigarette smoking.
§ Also adjusted for covariates potentially related to possible pathways of CV risk (i.e. blood pressure, TAG and total cholesterol:HDL-cholesterol ratio).
‖ Also adjusted for both covariates potentially related to possible pathways of CV risk and weight change.

Table 4. Sensitivity analyses excluding, one at a time, each level of Mediterranean Diet Score (MDS) 3†
(Hazard ratios (HR) and 95 % confidence intervals)

	All-cause mortality (*n* 553)		CVD				Myocardial infarction				Stroke			
			Incidence‡ (*n* 755)		Mortality (*n* 223)		Incidence‡ (*n* 161)		Mortality (*n* 64)		Incidence‡ (*n* 167)		Mortality (*n* 40)	
	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI
MDS recalculated excluding														
Fat ratio	0·92*	0·86, 0·98	0·91**	0·86, 0·96	0·85**	0·76, 0·94	0·91	0·80, 1·03	0·79*	0·65, 0·96	0·93	0·82, 1·05	0·92	0·71, 1·18
Vegetables	0·94	0·89, 1·01	0·92**	0·87, 0·97	0·88**	0·79, 0·97	0·92	0·82, 1·03	0·82*	0·68, 0·99	0·93	0·83, 1·04	0·98	0·77, 1·24
Fruit	0·96	0·90, 1·02	0·92**	0·87, 0·97	0·89*	0·80, 0·98	0·88*	0·78, 0·99	0·77**	0·64, 0·93	0·94	0·84, 1·05	1·04	0·82, 1·32
Cereal grains	0·92*	0·86, 0·98	0·92**	0·87, 0·97	0·88*	0·80, 0·97	0·89*	0·79, 1·00	0·80*	0·66, 0·96	0·95	0·84, 1·06	0·96	0·75, 1·22
Fish and shellfish	0·91**	0·86, 0·97	0·92**	0·87, 0·97	0·84***	0·76, 0·93	0·88*	0·78, 0·99	0·75**	0·62, 0·91	0·92	0·82, 1·03	0·92	0·72, 1·17
Wine	0·92**	0·87, 0·98	0·92**	0·87, 0·97	0·87**	0·79, 0·96	0·89*	0·79, 1·00	0·76**	0·63, 0·92	0·92	0·82, 1·03	0·98	0·78, 1·24
Dairy products	0·93*	0·87, 0·98	0·91***	0·87, 0·96	0·88**	0·80, 0·97	0·90	0·80, 1·01	0·85	0·71, 1·02	0·93	0·83, 1·04	0·96	0·76, 1·20
Meat products	0·92*	0·87, 0·98	0·92**	0·87, 0·97	0·87**	0·79, 0·96	0·90	0·80, 1·01	0·76**	0·63, 0·92	0·94	0·84, 1·05	0·99	0·78, 1·25

Values were significantly different: * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$.
† All results were obtained from a Cox proportional hazards model, adjusted for sex, BMI, education, physical activity and cigarette smoking.
‡ Fatal and non-fatal cases.

Table 5. Survival analyses on the association between the intake of single food groups above or below the median and several outcomes related to all-cause mortality and cardiovascular incidence and mortality†
(Hazard ratios (HR) and 95 % confidence intervals)

	All-cause mortality (*n* 553)		CVD				Myocardial infarction				Stroke			
			Incidence‡ (*n* 755)		Mortality (*n* 223)		Incidence‡ (*n* 161)		Mortality (*n* 64)		Incidence‡ (*n* 167)		Mortality (*n* 40)	
Single MDS component	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI
Intake above the median														
Fat ratio	0·93	0·79, 1·10	0·92	0·80, 1·06	0·92	0·71, 1·20	0·76	0·55, 1·03	0·68	0·41, 1·11	0·90	0·67, 1·22	1·32	0·70, 2·51
Vegetables	0·81*	0·68, 0·96	0·88	0·76, 1·02	0·81	0·62, 1·06	0·73*	0·54, 1·00	0·58*	0·35, 0·96	0·94	0·69, 1·27	0·90	0·48, 1·68
Fruit	0·74***	0·62, 0·88	0·86*	0·75, 1·00	0·72*	0·55, 0·94	1·01	0·73, 1·38	0·85	0·52, 1·42	0·87	0·64, 1·18	0·59	0·31, 1·12
Cereal grains	0·97	0·82, 1·15	0·90	0·78, 1·04	0·76*	0·58, 1·00	0·90	0·66, 1·24	0·69	0·41, 1·16	0·82	0·60, 1·11	1·00	0·53, 1·89
Fish and shellfish	1·05	0·88, 1·24	0·90	0·78, 1·04	1·12	0·86, 1·46	1·02	0·75, 1·39	1·12	0·68, 1·85	1·01	0·74, 1·37	1·38	0·72, 2·63
Total alcohol	1·09	0·92, 1·29	1·14	0·98, 1·31	1·24	0·95, 1·62	0·98	0·72, 1·35	1·32	0·80, 2·20	1·33	0·98, 1·81	1·48	0·78, 2·81
Wine	1·00	0·84, 1·18	0·93	0·80, 1·07	0·91	0·70, 1·19	0·96	0·70, 1·32	1·03	0·63, 1·70	1·05	0·77, 1·43	0·89	0·47, 1·68
Intake below the median														
Dairy products	1·00	0·84, 1·18	1·02	0·89, 1·18	0·85	0·65, 1·11	0·90	0·65, 1·22	0·49**	0·30, 0·82	0·97	0·72, 1·33	1·06	0·56, 2·02
Meat products	0·96	0·81, 1·14	0·91	0·79, 1·06	0·87	0·67, 1·15	0·89	0·65, 1·23	1·06	0·64, 1·74	0·89	0·65, 1·22	0·82	0·43, 1·58

MDS, Mediterranean Diet Score.
Values were significantly different: * $P \leq 0{\cdot}05$, ** $P \leq 0{\cdot}01$, *** $P \leq 0{\cdot}001$.
† All results were obtained from a Cox proportional hazards model, adjusted for sex, BMI, education, physical activity and cigarette smoking.
‡ Fatal and non-fatal cases.

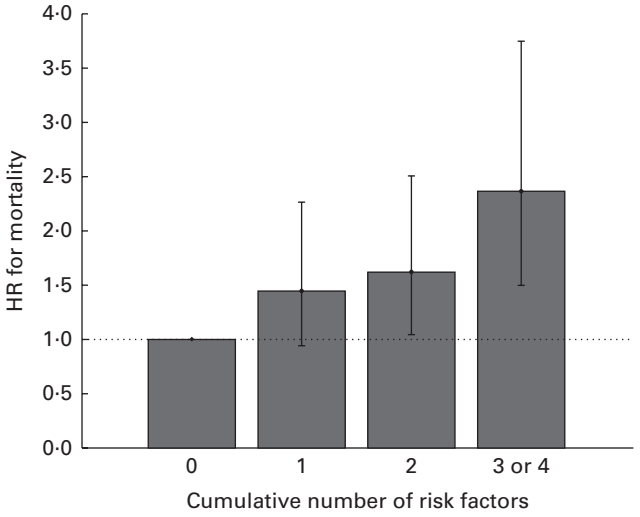

Fig. 1. Association between a cumulative number of risk factors related to both diet and lifestyle (including a modified Mediterranean Diet Score ≤4, ever being a smoker, low levels of physical activity and being obese) and all-cause mortality (adjusted by age). Values are hazard ratios (HR), with 95 % CI represented by vertical bars. *P* for trend <0·0001. Cumulative number of risk factors: 0 – *n* 144 (HR 1); 1 – *n* 606 (HR 1·45, 95 % CI 0·93, 2·26); 2 – *n* 787 (HR 1·62, 95 % CI 1·05, 2·51); 3 or 4 – *n* 312 (HR 2·37, 95 % CI 1·50, 3·75).

mortality was strong and significant (HR 2·37; 95 % CI 1·50, 3·75; *P* for trend <0·0001).

Discussion

The present study shows that the MDS was inversely associated both with all-cause mortality and with cardiovascular incidence and mortality, but not with stroke incidence and mortality. All associations proved to be robust to the adjustment for several potential confounders (i.e. sex, BMI, education, physical activity and smoking status) as well as covariates potentially related to possible pathways of CV risk, such as blood pressure, blood lipids and, notably, weight change after 11 years of follow-up. Increasing the number of unhealthy dietary or lifestyle-related factors additively increased the mortality risk by more than two times compared with those with no risk factors, as suggested by previous studies[29,30]. Fruit and vegetables were particularly relevant, as they attenuated the association of the diet score with some outcomes when excluded from score calculations.

Notably, only the MDS including ingredients from mixed dishes and recipes produced statistically significant results. It is not clear how ingredients have been treated in previous studies[9,29,31,32]. The present results imply that they should be included in the calculations of this score, because they allow for a more accurate depiction of a Mediterranean-like dietary pattern.

CVD prevention is of major public health importance, because of the negative impact of CVD on the quality of life[33] and the expected increase in the number of deaths[34]. Lifestyle changes and particularly dietary interventions are cost-effective means for the prevention of CVD and the reduction of the social and economic burden of this condition[35].

Several studies[13,29,32] have shown associations between the adoption of a Mediterranean dietary pattern and the lower risk of overall mortality and death because of IHD and CVD in general. Particularly, the MDS has been shown to be inversely associated with mortality in Swedish elderly[15] and in middle-aged Swedes where an inverse association with cardiovascular mortality (excluding stroke) was found in women and an association with cancer mortality was found in men[17]. In the latter study, fruit intake was inversely associated with all-cause mortality in women. The results of the present study again suggest that fruit and vegetable intakes can have particular importance in the reduction of cardiovascular risk. A few years ago, a meta-analysis of prospective studies had calculated a CHD risk reduction of 4 % for each additional portion of fruit and vegetables consumed per day[36]. However, the exact mechanisms by which these food items reduce CV risk are still unclear[37].

We do not have a clear explanation as to why the MDS and its single components were not at all associated with stroke mortality, also when separately analysing haemorrhagic *v.* non-haemorrhagic cases as well as ischaemic *v.* non-ischaemic cases. However, a tendency towards an inverse association was found for stroke incidence. Some epidemiological studies have also examined the association between a MDS and the risk of stroke. Among these, some[31,38,39] found an inverse association, while others did not[17,40]. Moreover, a recent paper has found an inverse association between adherence to a Mediterranean-like diet and total cerebrovascular disease[41]. Stroke mortality, in particular, could be more related to factors such as the use of anticoagulant therapy and the course of related infections, although the influence of the latter on stroke outcome is still debated[42].

Comparing the methodology used in the present study with the one that we had applied previously to elderly Swedes (based on a refined version of the MDS[15]), here a further score refinement was possible by adding the components of mixed dishes and recipes, which enabled us to identify the healthiest dietary pattern in relation to the health effects of a Mediterranean-like dietary pattern. Considering the alcohol component, in particular, one point should be noted. Based on the known U-shaped relationship between alcohol intake and CHD, previous studies using the MDS have given one point for moderate alcohol intakes (<50 g/d in men and 25 g/d in women) instead of negative scoring for high intakes[43,44]. However, since in our cohort only about 10 % of the subjects exceeded these thresholds, we decided to categorise alcohol intakes according to medians, as for the other food items.

The strengths of the present study include the adjustment of the analyses for several potential confounders as well as for covariates potentially related to possible pathways of cardiovascular risk such as blood pressure and lipids, our ability to examine effects on both MI and stroke, and the availability of a detailed food database based on a validated dietary assessment method that provided details of ingredients from mixed dishes and recipes. Additionally, there is almost 100 % follow-up for endpoints in the Danish registries as well as 100 % coverage of the Danish population[45], which means a

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negligible loss to follow-up. However, some limitations must also be acknowledged, such as possible residual confounding due to physical activity, as well as the likelihood that diets could have been misreported. However, random misreporting would have resulted in attenuation and not inflation of the hazards, suggesting that the observed significant association is likely to be true.

In conclusion, the present Danish population study demonstrates that within a Nordic setting, a high MDS is associated with longer survival and with a reduction in cardiovascular mortality, particularly, MI. Fruit and vegetable intakes seem to be of particular importance in influencing the strength of this association. Thus, the study supports evidence that the Mediterranean diet may be beneficial to health even among populations outside the Mediterranean basin. This is of importance considering that in Europe this pattern is not so common any more among the young generation[46].

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Does the Mediterranean diet predict longevity in the elderly? A Swedish perspective

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Abstract Dietary pattern analysis represents a useful improvement in the investigation of diet and health relationships. Particularly, the Mediterranean diet pattern has been associated with reduced mortality risk in several studies involving both younger and elderly population groups. In this research, relationships between dietary macronutrient composition, as well as the Mediterranean diet, and total mortality were assessed in 1,037 seventy-year-old subjects (540 females) information. Diet macronutrient composition was not associated with mortality, while a refined version of the modified Mediterranean diet index showed a significant inverse association (HR=0.93, 95% CI: 0.89; 0.98). As expected, inactive subjects, smokers and those with a higher waist circumference had a higher mortality, while a reduced risk characterized married and more educated people. Sensitivity analyses (which confirmed our results) consisted of: exclusion of one food group at a time in the Mediterranean diet index, exclusion of early deaths, censoring at fixed follow-up time, adjusting for activities of daily living and main cardiovascular risk factors including weight/waist circumference changes at follow up. In conclusion, we can reasonably state that a higher adherence to a Mediterranean diet pattern, especially by consuming wholegrain cereals, foods rich in polyunsaturated fatty acids, and a limited amount of alcohol, predicts increased longevity in the elderly.

Keywords Elderly · Mediterranean diet · Diet quality · Macronutrient intake · Diet adherence · Mortality

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Introduction

Epidemiologic studies indicate that quality of diet together with other lifestyle factors have considerable influence on health status as well as mortality risk (Huijbregts et al. 1997; Seymour et al. 2003; Haveman-Nies et al. 2003). Although many studies have examined effects of single nutrients, foods, or food groups on health status, it is becoming increasingly evident that the assessment of dietary patterns could be a practical alternative in the study of diet and health associations (Mai et al. 2005). Among other a priori patterns (such as the Healthy Eating Index or the Recommended Food Score), the Mediterranean diet has already been shown to possess many beneficial effects, as first became evident years ago in the Seven-Country Study (Keys 1980). This diet is characterized

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by a high intake of vegetables, legumes, fruits, nuts and seeds, cereals (that in the past were largely unrefined), and a high intake of olive oil combined with a low intake of saturated lipids, a moderately high intake of fish (depending on the proximity of the sea), a low-to-moderate intake of dairy products (and then mostly in the form of cheese and yogurt), a low intake of meat and meat products as well as a regular but moderate intake of ethanol, primarily in the form of wine and generally during meals (Willett et al. 1995).

The Mediterranean diet was first considered a dietary pattern that protected against coronary heart disease (de Lorgeril et al. 1999). This was confirmed very recently by a longitudinal study showing that Mediterranean diet is associated with a higher preservation of ventricular function and a more favorable prognosis after an acute coronary event (Chrysohoou et al. 2010). However, in previous studies, other beneficial effects on total mortality have been discovered (Trichopoulou et al. 2005). Interestingly, a recent paper on the Swedish population showed a reduction in total mortality among young women (Lagiou et al. 2006). In addition, a Mediterranean dietary pattern has also been shown to increase longevity among European elderly of the Healthy Aging: a Longitudinal study in Europe (HALE) project (Knoops et al. 2004).

In the present study, we investigated the association of the Mediterranean diet pattern with total mortality in a population of Swedish 70 year olds.

Subjects and methods

Subjects, dietary assessment, and outcome definition

This study is based on the Gerontological and Geriatric Population Studies in Gothenburg (H70; Eiben et al. 2004, 2005; Rothenberg et al. 1996, 1997, 1998). The Revenue Office of Gothenburg has a registry of all inhabitants, from which representative study groups of specific ages may be identified. Using this registry, population-based samples of 70-year-old residents were recruited for health examinations in 1971, 1981, 1992, and 2000. In the first two examinations, 70-year-old cohorts born in 1901 and 1911 were sampled and invited to participate based on date of birth. In the latter two examinations, men born in 1922 and 1930 were sampled in the same way, while the 70-year-old women born in those years were identified on the basis of previous inclusion in the Prospective Population Study of Women in Gothenburg (PPSW, Bengtsson et al. 1997; Lissner et al. 2003). Response rate decreased from 84% and 86% for men and women, respectively, in the earliest birth cohort to 65% for both sexes in the most recent cohort. Overall, a total of 1,277 men and women still residing in the Gothenburg district had undergone diet history examinations at the age of 70 (Eiben et al. 2004, 2005). Diet history was validated by comparison of energy intake (EI) with estimated total energy expenditure (TEE) by heart rate monitoring, activity diary, and doubly-labeled water (Rothenberg et al. 1998).

In order to exclude implausible dietary intakes, subjects in the highest and lowest 5 percentiles of the ratio between energy intake and calculated basal metabolic rate (BMR) were not included in the present analyses (N=149). BMR was predicted from weight (W) by means of standard equations for subjects aged 60–74 (males: 0.0499×W+2.93; females: 0.0386×W+2.875; Department of Health 1991). Other exclusions included cases missing information on diet or on potential confounders (N=91). The final study sample included 1,037 subjects (52.1% females). All cohorts have been monitored continuously for mortality by linking personal identification numbers with the national death registration system. The most recent mortality follow-up was in 2009. In a sub-sample of 781 subjects, a follow up at 75 years for both weight and waist circumference was available. In supplementary analyses, observation time was truncated to 8.5 years to give all cohorts equal follow-up time when estimating survival probabilities.

Definitions of food groups and of the Mediterranean diet scores

To assess the association of diet and lifestyle factors with total mortality, a refined version of the modified Mediterranean diet score (refined mMDS) was calculated, based on existing knowledge from the scientific literature on positive effects of wholegrain cereals (Flight and Clifton 2006) and moderate alcohol intake (Mukamal et al. 2010) as well as on the fact that polyunsaturated fats (PUFA) and not only monounsaturated fats (MUFA) are the principal unsaturated fats in non-Mediterranean diets (De Lorgeril et al.

1994). The score comprised nine components: (1) vegetables and potatoes, (2) legumes, nuts, and seeds, (3) fruit and fresh juices, (4) wholegrain cereals, (5) fish and fish products, (6) ratio of MUFA+PUFA to saturated fats (SAFA), (7) alcohol intake, (8) meat, meat products, and eggs, (9) dairy products. Intake of each component was adjusted to daily energy intakes of 2,500 kcal (10.5 MJ) for men and 2,000 kcal (8.5 MJ) for women. Although these values are not considered as recommended daily energy intake for both genders, they were chosen based on previous research on this subject (Knoops et al. 2004) and used to obtain energy adjusted associations in all the analyses. The sex-specific median intakes were taken as cut-off points. In any case, the final score is considered a measure of relative adherence to the Mediterranean diet pattern. The diet score varied from 0 (low-quality diet) to 9 (high quality diet). For each of the nine components, except for meat and dairy products, a value of 1 was assigned to subjects whose consumption was higher than the sex-specific median, and 0 to the others. For meat and dairy products, the reverse rule was applied. An alternative version of this score, previously used in the HALE project (HALE mMDS, Knoops et al. 2004) was also produced, including only MUFA in the numerator of the fat ratio, total instead of wholegrain cereals, and excluding alcohol intake from the score.

Statistical analyses

Cox proportional hazards models were used to estimate the effects of diet, smoking status, education, physical activity, and other potential confounders on total mortality, to November 2009. All the models used in this study were adjusted for potential confounders such as sex, baseline body mass index (BMI), waist circumference, smoking status, physical activity level, marital status, education, and birth cohort.

Analyses of macronutrients and mortality were first performed by means of the standard multivariate model (Willett 1998), in which each macronutrient was tested separately, with or without adjustment for energy intake. Further analyses were conducted by means of the multivariate nutrient density model (Willett 1998), in which the nutrient densities (percentages of energy from non-alcohol sources in a scale of 5% units) from total fat and protein intakes are included as independent variables. The model thus estimates the effect on the outcome of the iso-energetic replacement of 5% of energy provided by the reference macronutrient (the one not included in the model, e.g., carbohydrates) with a similar amount of calories provided by all the other macronutrients included in the model (e.g., proteins and fats). In these analyses, alcohol intake was added separately on a g/day basis.

Next, analyses were performed both on the Mediterranean diet scores (HALE and refined mMDS) on a continuous scale as well as on scores of 6–9 in refined mMDS or 5–8 in HALE mMDS in comparison with the others. In order to examine if the estimates for mortality were influenced by any of the covariates, or if any of these could remove part of the effect, the crude hazard ratios (HRs) for the dietary and lifestyle factors were also calculated and compared with the adjusted HRs. Finally, the mortality risk was also estimated in single food groups contributing to these scores. Food intakes were re-scaled according to medians, as done for the score calculations. For illustration, the dose–response effect of the mortality risk in subjects with growing levels of refined mMDS compared to the risk in subjects characterized by a score ≤ 1 was investigated.

In addition, a series of sensitivity analyses were performed, the most critical of which was the estimation of the association between mortality risk and different versions of the refined mMDS, calculated by excluding, one at a time, each component of the score in order to determine that the effect was not driven by a single food group. Other sensitivity analyses included adjusting for cardiovascular risk factors such as weight and waist circumference change, baseline blood pressure, fasting glucose, total cholesterol, and triglycerides or for activities of daily living variable (ADL, based on need of help for everyday activities). The activities of daily living variable was based on the Katz index, which is similar to the Barthel Index, and hence based on the need of help for at least one out of nine different activities, including house cleaning, shopping, transport, cooking, washing, dressing, using the toilet, ambulation, and feeding (Sonn and Asberg 1991). Furthermore, analyses were repeated after excluding the first 2 years of total mortality follow-up.

We also repeated the Cox analysis on the refined mMDS, by censoring at a fixed follow-up duration of 8.5 years (approximately the longest follow-up time in the latest-born cohort) instead of censoring at a fixed date. Crude proportions of subjects still alive at 8.5 years were also calculated according to three

different levels of the score (≤3, 4–6, ≥7) and the association between adherence to the Mediterranean diet pattern and survival was assessed by means of a chi-square test.

Finally, potential confounders (see above) were examined in more detail, including an estimation of the mortality HRs in subjects characterized by a score in the highest four levels (>4 for the HALE mMDS, >5 for the refined mMDS) compared to the other subjects. Moreover, the assessment of the effect of all main covariates on mortality included in the previous models was also performed.

All statistical analyses were considered significant under a p value of 0.05 and were carried out using the SAS statistical software version 9.2 (SAS Institute, Cary, NC, USA).

Bioethics

The latest H70 examinations (since 1992) have been approved by the Gothenburg University Ethics Committee in accordance with the Declaration of Helsinki (1989) of the World Medical Association. All participants were informed of the aims and procedures of the study and gave their consent.

Results

Descriptive analyses

Main descriptive analyses are reported in Tables 1 (dietary variables), 2 (Mediterranean diet scores), and 3 (potential confounders and other cohort descriptors). Dietary intake distributions across the birth cohorts have previously been described (Eiben et al. 2004; Rothenberg et al. 1996). Table 1 shows that, according to trend test, across the four birth cohorts there was an increase in the consumption of vegetables and potatoes, fruit, legumes plus nuts and seeds, fish and fish products, meat and meat products, red wine and alcohol. In contrast, a decrease in the consumption of cereals (both total and wholegrain) was observed. No clear patterns emerged for dairy products, MUFA/SAFA ratio and (MUFA+PUFA)/SAFA ratio. Regarding macronutrient intakes, a trend in protein, carbohydrate, and fat intake was observed. Energy intakes showed a slight tendency to an increase across the cohorts, and the ratio between energy intake and BMR was consistently high (1.5±0.3), indicating a good quality of nutritional data. In the subjects excluded for implausible dietary intakes (highest and lowest 5 percentiles of energy intake vs. basal metabolic rate ratio), EI/BMR was either less than 0.9 or greater than 2.2 (data not shown).

Regarding the two scores measuring the adherence to the Mediterranean diet (Table 2), no major differences appeared from cohort to cohort, although for the refined mMDS a slight but significant increase was evident (4.3±1.6 in 1901 cohort to 4.8±1.8 in 1930 cohort), probably due to the increase in wholegrain, alcohol, and PUFA intakes included in this score and not in the HALE mMDS.

Table 3 shows the descriptive analyses of potential confounders. Both genders were equally represented in the population (47.9% men). Subjects characterized by a BMI <20 were only a minority (3.1%), while obese subjects constituted 15.8% of the total population. A high waist circumference (>88 cm in women, >102 cm in men), characterized 31.0% of the subjects. Only 14.2% of subjects were physically inactive, 62.1% were married, and more than 30% of subjects were smokers (or stopped less than 10 years ago) or had an education above basic (i.e., 6 years of schooling or more). Overall, in the studied population, there were 630 deaths (60.8%) until November 2009: 309 women and 321 men.

Regarding main cardiovascular risk factors depicted in Table 3, which include variations in anthropometry over 5 years, we observed a decrease in mean weight from 74.2 kg±12.9 at baseline to 72.3 kg±13.2 at follow up (mean change: −1.9 kg±5.4), a quite stable waist circumference (mean change: −0.2 cm±7.4) and BMI (mean change: −0.3 kg/m^2±1.9). Diastolic blood pressure means were less than 90 mmHg (87.4 mmHg±11.9), while systolic blood pressure mean was above 140 mmHg (159.0 mmHg±23.6). Regarding plasma parameters (only available at baseline), fasting glucose was equal to 100.8 mg/dL±36.0, total cholesterol was 233.3 mg/dL±77.8 while triglycerides was 132.4 mg/dL±70.6.

Diet quality and mortality

In the present cohort, the total mortality was around 60%, with a higher rate in the earlier born cohorts and a lower in the later born cohorts (100% in 1901, 98% in 1911, 48% in 1922, and 15% in 1930 birth cohort).

Table 1 Means and standard deviation in comparison with medians and 95% Confidence Limits (CLs) of dietary variables used in the analyses, including test for trend across the birth cohorts

Food groups/macronutrients	Overall mean (N=1,037)	Median intakes and 95% CLs	p for trend across birth cohorts[e,f]
Vegetables and potatoes (g/day)[a,b]	237.6±98.7	♀: 209.5 (99.6; 406.3) ♂: 239.0 (120.1; 432.2)	<0.0001
Fruit (g/day)[a,b]	196.6±146.3	♀: 176.4 (22.3; 527.7) ♂: 155.5 (14.0; 456.0)	<0.0001
Legumes nuts and seeds (g/day)[a,b]	15.2±20.2	♀: 2.0 (0; 40.0) ♂: 13.3 (0; 60.0)	<0.0001
Cereals (g/day)[a]	207.4±104.0	♀: 165.0 (68.2; 383.0) ♂: 213.0 (98.3; 442.1)	(−)<0.0001
Wholegrain cereals (g/day)[b]	107.9±95.6	♀: 74.2 (0; 298.5) ♂: 92.8 (0; 322.0)	(−)<0.0001
Fish and fish products (g/day)[a,b]	53.8±35.8	♀: 45.2 (12.8; 105.3) ♂: 53.7 (13.8; 129.5)	<0.0001
Dairy products (g/day)[a,b]	445.1±251.7	♀: 373.3 (127.9; 829.5) ♂: 446.0 (74.0; 1061.9)	0.41
Meat and meat products (g/day)[a]	105.4±47.9	♀: 89.7 (38.4; 168.8) ♂: 109.1 (52.5; 204.9)	<0.0001
Meat, meat products, eggs (g/day)[b]	129.5±55.3	♀: 110.1 (47.9; 187.3) ♂: 137.7 (66.3; 251.1)	<0.001
Red wine (g/day)[c]	2.0±4.7	♀: 0 (0; 9.8) ♂: 0 (0; 10.7)	<0.001
Alcohol (g/day)[b]	6.0±9.1	♀: 1.3 (0; 13.9) ♂: 5.3 (0; 28.5)	<0.0001
Carbohydrate (g/day)[d]	249.1±64.8	♀: 217.1 (143.5; 316.8) ♂: 272.4 (192.8; 398.2)	0.01
Protein (g/day)[d]	80.1±20.0	♀: 71.2 (48.6; 100.4) ♂: 85.2 (58.3; 129.0)	<0.0001
Fat (g/day)[d]	86.1±25.4	♀: 74.6 (46.6; 110.) ♂: 93.5 (60.1; 146.5)	<0.0001
MUFA/SAFA[a]	0.8±0.2	♀: 0.8 (0.6; 1.1) ♂: 0.8 (0.6; 1.1)	0.40
(MUFA+PUFA)/SAFA[b]	1.2±0.3	♀: 1.1 (0.8; 1.6) ♂: 1.1 (0.7; 1.7)	0.50
Energy intake (100 kcal)	21.4±5.0	♀: 18.5 (13.3; 25.4) ♂: 23.6 (17.7; 33.6)	0.04
Energy/BMR[d]	1.5±0.3	♀: 1.4 (1.0; 1.9) ♂: 1.4 (1.1; 2.1)	0.30

[a] Included in HALE mMDS

[b] Included in refined mMDS

[c] Included in place of alcohol in an additional analysis

[d] Not included in any scores, just included for descriptive purposes

[e] Birth–cohort effect from a regression model adjusted for gender, BMI, waist circumference, physical activity, smoking status, marital status and education

[f] All significant trends positive in direction except if indicated with (−)

In the first part of the present work, we analyzed the association between diet macronutrient composition and total mortality. No clear association emerged either when analyzing protein, fat, and carbohydrate intake (adjusted or unadjusted for energy intake) in standard multivariate models. Alcohol was also not associated on a continuous scale with total survival time (Table 4). Similar results were obtained when

Table 2 General descriptive of Mediterranean diet score distributions, both stratified by birth cohort and for the overall population

Mediterranean diet score	1901 (*N*=323)	1911 (*N*=214)	1922 (*N*=88)	1930 (*N*=412)	Overall (*N*=1,037)	*p* for trend across birth cohorts[a]
Refined mMDS (mean±SD)	4.3±1.6	4.0±1.6	4.8±1.5	4.8±1.8	4.5±1.7	<0.001
Medians (5th;95th perc)	4 (2; 7)	4 (1; 7)	5 (2; 7)	5 (2; 8)	4 (2; 7)	
HALE mMDS (mean±SD)	4.1±1.5	3.6±1.4	4.1±1.4	4.1±1.6	4.0±1.5	0.02
Medians (5th;95th perc)	4 (2; 6)	4 (1; 6)	4 (2; 6)	4 (1; 7)	4 (2; 6)	

[a] Birth–cohort effect from a regression model adjusted for gender, BMI, waist circumference, physical activity, smoking status, marital status, and education

employing the nutrient density model to describe the effect of the reciprocal substitution of 5% of energy intake from each macronutrient with carbohydrates (Table 4).

Table 3 Main covariate frequencies and cohort description

Potential confounders	Frequency (%)
Total sample (*N* = 1,037)	
Male gender	47.9
BMI<20	3.1
BMI>30	15.8
High waist circumference	31.0
Low physical activity at 70	14.2
Married at age 70	62.1
Smoker/stopped <10 years ago	30.6
School education above basic	30.5
Activities of daily living (ADL)	12.7
Additional covariates	Means±SD
Baseline diastolic blood pressure (mmHg)	87.4±11.9
Baseline systolic blood pressure (mmHg)	159.0±23.6
Baseline fasting glucose (mg/dL)	100.8±36.0
Baseline total cholesterol (mg/dL)	233.3±77.8
Baseline triglycerides (mg/dL)	132.4±70.6
Subsample with follow up at 75 years (*N* = 781)	
Changes in anthropometry	Means±SD
Weight change (kg)	−1.9±5.4
Baseline	74.2±12.9
Follow up	72.3±13.2
Waist circ. change (cm)	−0.2±7.4
Baseline	90.0±11.4
Follow up	90.0±11.8
BMI change (kg/height^2)	−0.3±1.9
Baseline	26.4±3.9
Follow up	26.1±4.1

In light of the null results obtained by the analyses of macronutrients, we then focused on the identification of a possible dietary pattern, which could be related to total mortality. In particular, we chose to study the Mediterranean diet pattern by means of a refined version of the modified Mediterranean Diet Score (refined mMDS), which we consider to reflect a closer adherence to the classic Mediterranean diet pattern.

Table 5 shows the association of refined mMDS, in comparison to the previously used HALE mMDS (Knoops et al. 2004) with mortality risk, as well as the same outcome for those food groups on whose intakes the adherence to Mediterranean diet pattern was assessed.

Regarding Mediterranean diet score, an inverse association with total mortality was shown for the continuous refined mMDS (HR=0.93, 95% CI: 0.89; 0.98), while no significant association emerged with HALE mMDS, although the trend was toward an inverse association (HR=0.97, 95% CI: 0.92; 1.02). Moreover, the comparison of the lowest-risk group

Table 4 Hazard ratios and 95% confidence limits from a Cox-proportional hazard model (adjusted for gender, baseline BMI, waist circumference, physical activity, marital status, smoking status, birth cohort, and education) estimating the association of macronutrient with mortality risk and different substitution of energy from each macronutrient with the same amount of energy of another one

Macronutrient (10 g)	HR	95% CLs
Alcohol	0.98	0.86; 1.11
Protein	0.97	0.90; 1.04
CHO	1.00	0.98; 1.03
Fat	1.00	0.94; 1.07
From CHO to protein 5% energy	0.95	0.80; 1.14
From CHO to fat 5% energy	1.01	0.94; 1.09

Table 5 Hazard ratios and 95% confidence limits from a Cox-proportional hazard model (adjusted for gender, baseline BMI, waist circumference, physical activity, marital status, smoking status, birth cohort, and education) estimating the association of both HALE and refined mMDS with mortality risk

Mediterranean diet score or food group	HR	95% CLs
Refined mMDS	0.93	0.89; 0.98
Crude estimate	0.92	0.88; 0.97
Highest 4 levels vs. the others	0.82	0.67; 0.99
Crude estimate	0.81	0.67; 0.99
HALE mMDS	0.97	0.92; 1.02
Crude estimate	0.97	0.92; 1.03
Highest 4 levels vs. the others	0.94	0.79; 1.11
Crude estimate	0.94	0.80; 1.11
High intake/level of:		
Vegetables and potatoes	1.06	0.90; 1.24
Fruit	1.03	0.87; 1.21
Legumes, nuts, and seeds	0.98	0.83; 1.16
Cereals	1.01	0.86; 1.19
Wholegrain cereals	0.85	0.73; 1.00
Fish	0.96	0.82; 1.13
Alcohol	0.77	0.61; 0.97
MUFA/SAFA ratio	0.98	0.84; 1.15
(MUFA+PUFA)/SAFA ratio	0.96	0.82; 1.13
Low intake of:		
Dairy products	0.82	0.70; 0.96
Meat and meat products	0.89	0.76; 1.05
Meat, meat products and eggs	0.84	0.71; 0.98

(highest four levels of the score) versus the other subjects showed a significant inverse association for the refined mMDS (HR=0.82, 95% CI: 0.67; 0.99), but not for the highest levels of the HALE mMDS (HR=0.94, 95% CI: 0.79; 1.11; Table 5). Crude estimates were also calculated and, as shown in the table, they are quite overlapping with the adjusted ones, thus showing that covariates did not have any strong influence on results. The protective effect of the Mediterranean diet pattern was stronger in the two youngest birth cohorts compared to the others (data not shown).

When studying the effect of single food groups, an inverse association with total mortality was shown for high intakes of wholegrain cereals (HR=0.85, 95% CI: 0.73; 1.00), alcohol consumption (HR=0.77, 95% CI: 0.61; 0.97) and low intake of dairy products (HR=0.82, 95% CI: 0.70; 0.96) as well as low intakes of meat, meat products, and eggs (HR=0.84, 95% CI: 0.71; 0.98). In contrast, other food groups were not significantly associated with mortality, such as vegetables and potatoes (HR=1.06, 95% CI: 0.90; 1.24), fruit (HR=1.03, 95% CI: 0.87; 1.21), legumes plus nuts and seeds (HR=0.98, 95% CI: 0.83; 1.16), and fish (HR=0.96, 95% CI: 0.82; 1.13). No association was shown for either of the fat ratios (MUFA/SAFA ratio: HR=0.98, 95% CI: 0.84: 1.15; (MUFA+PUFA)/SAFA ratio: HR=0.96, 95% CI: 0.82; 1.13). It is worth mentioning that vegetables alone and potatoes alone did not show any association with total mortality (data not shown).

In Fig. 1 dose–response analysis results are depicted. Briefly, the group characterized by a refined mMDS equal to 0 or 1 was the reference, compared to the other groups with increasing levels of the score. Although none of the single comparisons reached statistical significance, the analysis showed a dose–response tendency, thus suggesting a decrease in the mortality risk as long as the adherence to the Mediterranean diet pattern increased.

Supplementary analyses

A sensitivity analysis was performed on the refined mMDS, with the aim of understanding whether any one of the food group items included in the score could invalidate the effect of the entire score, with respect to mortality. Nine different scores were then produced, each excluding one item at a time (and keeping all others). Table 6 clearly demonstrates that the association of the refined mMDS with mortality is robust and survives all the item-by-item exclusions.

Furthermore, in order to test whether the positive effect of alcohol intake within the score could be obtained considering red wine (instead of total alcohol intake from all alcoholic beverages), considered a more accurate indicator of the Mediterranean alcoholic drinks, we ran an additional analysis, ending up with a similar outcome and a slightly stronger hazard ratio (HR: 0.92, 95% CI: 0.87; 0.97; Table 6).

In order to confirm that the effect of diet on mortality was independent of the subjects' functional capacity levels, and thus that the effect of Mediterranean diet was not entirely explained by how impaired the subjects were at the time of diet assessment we also repeated all the models adjusting for activities of daily living variable and found no substantial change in results. Similar analyses were performed adjusting for

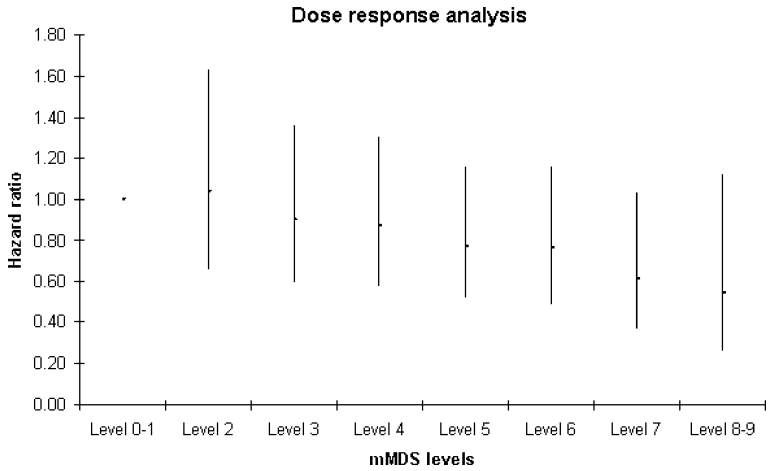

Fig. 1 Dose–response analyses based on Cox-proportional hazard model (adjusted for gender, baseline BMI, waist circumference, physical activity, marital status, smoking status, birth cohort and education) comparing mortality risk in subjects characterized by a refined mMDS ≤1 with the same risk in subjects in the other score categories. *Dots* and *vertical lines* indicate HR and 95% confidence limits

other cardiovascular risk factors, i.e., baseline blood pressure, fasting glucose, total cholesterol, and triglycerides; this did not strongly affect the results, except that the association of the dichotomous score (higher vs. lower) was attenuated (HR=0.85, 95% CI: 0.70; 1.04). Adjustment for weight or waist circumference change did not substantially affect the association of the refined mMDS with mortality. However, both were inversely associated with total mortality (weight change: HR=0.95, 95% CI: 0.93; 0.97; waist circumference change: HR=0.98, 95% CI: 0.97; 1.00), suggesting a greater mortality risk in association with weight loss. Furthermore, to minimize the possibility that diet or lifestyle factors had changed in response to subclinical diseases, all analyses were also repeated after exclusion of the subjects who had died in the first 2 years of follow up. Here again, no clear difference emerged, thus confirming our main results.

Finally, the association of the refined mMDS with total mortality was also tested in a Cox model in which subject censoring was done at a fixed follow up time (8.5 years, the maximum in the youngest birth cohort) instead at a fixed date. The previous results were confirmed although somewhat attenuated (HR=0.92, 95% CI: 0.86; 1.00). The distribution of crude proportions of survival rates at 8.5 years, stratified by increasing levels of the refined mMDS were as follows: 67% survival among those characterized by a score ≤3,77% survival at intermediate levels of the score (from 4 to 6) and 80% survival among the subjects with a score ≥7 (p=0.02).

Table 6 Hazard ratios and 95% confidence limits from a Cox-proportional hazard model-based sensitivity analysis estimating the effect of the exclusion of each level of the refined mMDS+alcohol on mortality risk (adjusted for gender, baseline BMI, waist circumference, physical activity, marital status, smoking status, birth cohort, and education)

Mediterranean diet score	HR	95% CLs
Refined mMDS	0.93	0.89; 0.98
Without fat ratio	0.92	0.87; 0.97
Without vegetables and potatoes	0.91	0.87; 0.96
Without fruit	0.92	0.87; 0.97
Without legumes, nuts and seeds	0.93	0.88; 0.97
Without cereals	0.94	0.89; 0.99
Without fish	0.92	0.87; 0.97
Without dairy	0.94	0.89; 0.99
Without meat	0.94	0.90; 0.99
Without alcohol	0.94	0.89; 0.99
Refined mMDS (including red wine instead of alcohol)	0.92	0.87; 0.97

Effect modification and covariates associated with diet and mortality

In order to identify potential effect modifiers, statistical interactions between all dietary and potential confounders were tested, but none of them were statistically significant at a 0.05 level. The association of the highest levels of the refined mMDS with each of the above-mentioned confounders was also investigated (Table 7). Subjects ranked to the highest levels of the score were less likely to be inactive (OR=0.42; 95% CI: 0.25; 0.71) and more likely to be married (OR=1.47, 95% CI: 1.07; 2.01), more educated (OR=1.38, 95% CI: 1.02; 1.86) or born within the

Table 7 Hazard ratios and 95% confidence limits from a Cox-proportional hazard model estimating the association of potential confounders included in the final model and mortality risk in the whole studied population (N=1,037)

Confounders	Association with highest levels of refined mMDS		Association with mortality	
	OR	95% CLs	HR	95% CLs
Male gender	0.90	0.66; 1.23	2.02	1.67; 2.44
BMI<20	1.06	0.45; 2.50	1.32	0.90; 1.96
BMI>30	0.92	0.56; 1.51	0.94	0.72; 1.24
High waist circumference	0.80	0.54; 1.18	1.29	1.04; 1.60
Low physical activity at 70	0.42	0.25; 0.71	1.29	1.04; 1.59
Married at age 70	1.47	1.07; 2.01	0.78	0.66; 0.93
Smoker/stopped <10 years ago	1.07	0.77; 1.47	1.37	1.14; 1.64
School education above basic	1.38	1.02; 1.86	0.91	0.75; 1.11
Birth cohort	1.03	1.01; 1.04	0.97	0.97; 0.98

more recent birth cohorts (OR 0 1.03, 95% CI: 1.01; 1.04).

Table 7 also shows the association of the potential confounders included in the final model with total mortality. As expected, there is a positive association between the risk of mortality and male gender (HR= 2.02, 95% CI: 1.67; 2.44), high waist circumference (HR=1.29, 95% CI: 1.04; 1.60), low level of physical activity (HR=1.29, 95% CI: 1.04; 1.59) and smoking (HR=1.37, 95% CI: 1.14; 1.64). Inverse associations were found for marital status (HR=0.78, 95% CI: 0.66; 0.93), and for birth cohort, which we considered as a proxy for age/period effects (HR=0.98, 95% CI: 0.97; 0.98). Finally, BMI did not show any significant association.

Discussion

In this paper, we investigated the association of a dietary pattern close to the Mediterranean diet with total mortality in population-based cohorts of 70-year-old Swedes. Although we did not find an association when testing the previously defined HALE mMDS on our Swedish population (Knoops et al. 2004), we were able to refine this score in order to obtain one that best described strict adherence to a Mediterranean diet-like pattern. None of the items included in the score were found to be essential for the association with the total score. Finally, the association was not attenuated when adjusting for ADL, thus showing that the protection by the Mediterranean pattern was not limited to those subjects still living independently at the time of the dietary assessment, or biased in some way by how impaired they were at enrolment. Furthermore, the effect was only slightly attenuated when adjusting for several indicators of cardiovascular risk, suggesting that the protective effect was not limited to high-risk subjects.

Other studies had already found a positive association of Mediterranean diet and health (Lagiou et al. 2006; Knoops et al. 2004; Trichopoulou et al. 1995), showing the effects of increasing the intake of vegetables and fruit, fish, and cereals, while decreasing animal products such as dairy and meat. In our study, we started by reproducing the same mMDS that was previously created by the HALE working group for their paper on Mediterranean diet and total mortality (Knoops et al. 2004) and did not find a significant trend, even when testing the association at the highest levels. However, we found an inverse association with total mortality when a refined version of the mMDS was tested instead of the original score. The association was indeed strong and robust after calculating the score considering wholegrain cereals instead of total cereals, adding alcohol (or red wine) intake, considering egg intake together with meat products and calculating the fat ratio including PUFA and not only MUFA in the numerator. The addition of alcohol intake should not be considered problematic, since the intakes in this population were not high (75th percentile: 7.6 g/day, 90th percentile: 16.3 g/day) and can then be considered in an order of magnitude quite close to international recommendations. Interestingly, beneficial effects of moderate alcohol intake have been recently confirmed in a study with a follow up of

20 years that also considered many confounders typically associated with abstaining (such as past history of heavy drinking behavior, Holahan et al. 2010). Furthermore, our association was also confirmed when including red wine instead of alcohol, confirming that the positive result characterizes the subjects adhering to the healthiest diet patterns.

The possible explanation of the necessity of improving an existing score that had already worked in previous studies to find a robust association in the Swedish elderly may be due to the obvious differences between the original Mediterranean diet and the Swedish Mediterranean-like diet. This is particularly important in our population, since many subjects were born at the beginning of last century, when many products that were common in Southern Europe were not consumed in large quantities in the north. Swedish food habits have indeed undergone major changes in the last 30 years, particularly in relation to consumptions of fresh fruit and vegetables (now available in quantity even on the Scandinavian market) and cereals as well as a higher unsaturated fat proportion (Eiben et al. 2004). Another reasonable example is represented by MUFA sources, mainly olive oil in Southern European countries, mostly margarines (a source of trans fatty acids, especially at the time they were measured) in the oldest birth cohorts and only marginally derived from olive oil in the 1930 birth cohort.

However, by applying stricter rules, such as substituting wholegrain cereals instead of total cereals (often characterized by a higher sugar content than in South Europe), including PUFA intake (in Sweden, fish is an important contributor), adding alcohol (and also red wine only), and including egg intake (a probable indicator of a low-quality diet), we found a protective pattern in our Swedish population.

Another important issue is that single food groups were not always found to be inversely or directly associated with the mortality risk, although the full score is robust to exclusions of any single component. This suggests that the use of this and similar scores in nutritional epidemiology studies is indeed a useful strategy for investigating associations between diet and health outcomes.

Our study has both strengths and limitations, the former being the high quality of nutritional data obtained by a validated diet history during a face to face interview with the dietician (confirmed by the high EI/BMR ratio and by concordance of our estimated intakes with National Surveys; Becker 1994), the possibility of adjusting for different known confounders and the availability of weight and waist circumference measurements both at baseline and at follow up. The limitations include the lack of repeated dietary assessment and the small size of the study population. Moreover, it must be acknowledged that diet patterns alone may inherently co-vary with other health-related phenomena (e.g., healthy lifestyles, illnesses, weight status). Thus, it is important to note that associations were independent of cardiovascular risk factors, weight and waist circumference change, SES, education, ADL, and other risk factors. Moreover, it is worth mentioning that although the analyses were always adjusted for birth cohort, the results could still be influenced by a residual cohort effect as suggested by the fact that the protective effect of the Mediterranean diet pattern was stronger in the youngest cohorts. This was probably due to a higher quality of nutritional data (or higher health consciousness in the subjects) in the most recent surveys.

From the above mentioned results, it clearly emerges that it would be useful to put more emphasis on dietary recommendations directed to the elderly in order to encourage increased consumption of fruit and vegetables, wholegrain cereals and fish, while reducing the intake of dairy and meat products, in favor of other healthier protein sources such as legumes. This is particularly important in Sweden, considering that current guidelines do not always place sufficient emphasis on this type of recommendation. For instance, although our results are quite in line with Nordic Nutrition Recommendations (Nordic Council of Ministers 2004), the present Swedish Nutrition Recommendations Objectified (Barbieri and Lindvall 2005), is in some ways different from Mediterranean diet-based features. Indeed, the former considers acceptable a daily intake of 500 ml milk (in addition to other dairy products) and suggests some equilibrated menus that do not consider olive oil either as a condiment or as a cooking fat, while including margarine on a daily basis. Moreover, it often refers to "refined rye bread" and "white bread" instead of suggesting wholegrain products, and includes meat products in two meals per day. At the same time, it is also worth mentioning that carbohydrate restriction is becoming an increasingly popular (although not scientifically based) weight control method in the population, a modification which is also not supported, vis-à-

vis mortality, in our nutrient-level analyses simulating carbohydrate replacement.

Overall, studies of dietary patterns are inherently complex. However, regardless of scientific approach, there is a remarkable convergence of evidence on the fact that dietary patterns associated with longevity emphasize fruits and vegetables and are reduced in saturated fat, meats, refined grains, sweets, and full-fat dairy products (Appel 2008). This is not only the case for the Mediterranean diet pattern, but also for other pattern such as the DASH diet (Parikh et al. 2009) or the Okinawa diet (Willcox et al. 2009).

Conclusions

To conclude, we can reasonably state that the adherence to a Mediterranean-like dietary pattern is inversely related to total mortality also in a Swedish population of elderly subjects. Our hope is that the results of the present research will stimulate a productive discussion on these issues and be considered in updated food- and nutrient-level guidelines for the population.

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Nonfermented milk and other dairy products: associations with all-cause mortality[1,2]

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ABSTRACT

Background: A positive association between nonfermented milk intake and increased all-cause mortality was recently reported, but overall, the association between dairy intake and mortality is inconclusive.

Objective: We studied associations between intake of dairy products and all-cause mortality with an emphasis on nonfermented milk and fat content.

Design: A total of 103,256 adult participants (women: 51.0%) from Northern Sweden were included (7121 deaths; mean follow-up: 13.7 y). Associations between all-cause mortality and reported intakes of nonfermented milk (total or by fat content), fermented milk, cheese, and butter were tested with the use of Cox proportional hazards models that were adjusted for age, sex, body mass index, smoking status, education, energy intake, examination year, and physical activity. To circumvent confounding, Mendelian randomization was applied in a subsample via the lactase *LCT-13910 C/T* single nucleotide polymorphism that is associated with lactose tolerance and milk intake.

Results: High consumers of nonfermented milk (≥2.5 times/d) had a 32% increased hazard (HR: 1.32; 95% CI: 1.18, 1.48) for all-cause mortality compared with that of subjects who consumed milk ≤1 time/wk. The corresponding value for butter was 11% (HR: 1.11; 95% CI: 1.07, 1.21). All nonfermented milk-fat types were independently associated with increased HRs, but compared with full-fat milk, HRs were lower in consumers of medium- and low-fat milk. Fermented milk intake (HR: 0.90; 95% CI: 0.86, 0.94) and cheese intake (HR: 0.93; 95% CI: 0.91, 0.96) were negatively associated with mortality. Results were slightly attenuated by lifestyle adjustments but were robust in sensitivity analyses. Mortality was not significantly associated with the *LCT-13910 C/T* genotype in the smaller subsample. The amount and type of milk intake was associated with lifestyle variables.

Conclusions: In the present Swedish cohort study, intakes of nonfermented milk and butter are associated with higher all-cause mortality, and fermented milk and cheese intakes are associated with lower all-cause mortality. Residual confounding by lifestyle cannot be excluded, and Mendelian randomization needs to be examined in a larger sample. *Am J Clin Nutr* doi: 10.3945/ajcn.116.140798.

Keywords: all-cause mortality, butter, cheese, dairy products, fermented dairy products, fermented milk, milk, nonfermented milk

INTRODUCTION

Milk and other dairy products constitute important sources of energy as well as macronutrients and micronutrients in most Western countries, but intakes vary largely between populations (1). Associations between dairy intake and different disease outcomes have been evaluated in several studies, but reported associations remain contradictory (2–10). The inconclusive evidence regarding the association between nonfermented milk, specifically, and mortality has been highlighted in a recent systematic review and meta-analysis by Larsson et al. (3). The authors concluded that "large prospective studies assessing the relation between milk consumption and mortality are warranted."

In Sweden, intake of dairy products is among the highest in the world (11, 12) and is concurrent with a low prevalence of lactose intolerance (13). According to 2011 food-balance sheets by the FAO, the average per-capita milk supply in Sweden was the third highest worldwide after that of Finland and the Netherlands (14). Traditionally, low-fat milk and dairy products have been promoted as healthy and nutritious food choices by the Nordic Nutrition Recommendations (15) and international authorities (16). In line with the recommendations, a marked drop in intake of high-fat nonfermented milk in favor of medium-fat alternatives was observed in Sweden between 1986 and 1991 (17). In recent years, fat intake from dairy products has increased in Sweden (17),

[1] Supported by the Swedish Research Council (VR) and the Swedish Research Council for Health, Working Life and Welfare (FORTE) [grants to The Northern Sweden Diet Database, including the Västerbotten Intervention Program and Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) data]. The Västerbotten Intervention study is supported by the County Council of Västerbotten, Sweden, and the MONICA screenings are supported by Norrbotten and Västerbotten County Councils, the VR, and the FORTE. GT received salary support from the FORTE (2006-1506, EpiLife center).

[2] Supplemental Tables 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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likely reflecting a strong promotion of low-carbohydrate, high-fat diets in the Swedish media (18). On the basis of the high consumption and wide range of intake of dairy products, Sweden offers a unique setting for studying the association of dairy intake with human health. Recently, a Swedish study by Michaëlsson et al. (19) presented a significant positive dose-response association between nonfermented milk intake and all-cause mortality for women and men, whereas an inverse association was observed for fermented dairy products. However, the study did not report the associations for nonfermented milk stratified by the fat content.

Observational nutritional studies are prone to confounding, reverse causality, and bias. To overcome confounding in observed associations between nonfermented dairy intake and all-cause mortality, the single nucleotide polymorphism (SNP)[11] *LCT-13910 C/T* (20) that predisposes individuals to lactose intolerance can be used as an instrumental variable for milk intake within the Mendelian randomization framework (21). Individuals with the CC genotype are lactose intolerant (lactase nonpersistent) with less ability to tolerate milk than are individuals with the lactase-persistent TT/TC genotypes, making this SNP an unbiased proxy exposure for milk consumption.

In the current article, we aimed to extend the analysis by Michaëlsson et al. (19) and study the association between intake of dairy products and all-cause mortality with special emphasis on reported intake of nonfermented milk in total and by fat content in >100,000 men and women in a large population-based cohort from Northern Sweden [The Northern Sweden Health and Disease Study (NSHDS)] and to apply Mendelian randomization in an attempt to reduce the effect of confounding.

METHODS

Study population and design

Participants in the Västerbotten Intervention Program (VIP) and the Northern Sweden Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study (both within the NSHDS) with available dietary information were eligible for the current study.

The VIP, which was initiated in 1985 and is still ongoing, runs in the county of Västerbotten in Northern Sweden with ~260,000 inhabitants of whom ~121,000 individuals live in the main city of Umeå. In the VIP, residents of the county are invited to a health examination when they turn 40, 50, and 60 y of age, and some communities have also invited 30-y-olds. Approximately 30% of individuals have a 10-y follow-up visit. Participants undergo an extensive health examination including measurements of anthropometric measures, blood pressure, and blood lipid profiles, an oral-glucose-tolerance test, and an extensive questionnaire on diet, lifestyle, and health conditions. The mean recruitment rate has been ~60% of available participants, and only limited evidence of selection bias in relation to income, age, and unemployment has been reported (22). No difference was observed in cancer incidence in the VIP cohort compared with in the population of Västerbotten at large, thereby providing further evidence that the VIP is a representative population cohort (23).

In the Northern Sweden MONICA study, cross-sectional samples of residents in the counties of Västerbotten and Norrbotten have been randomly selected from updated population registers every fourth to fifth year (24). Sampling was stratified to select equal numbers by sex and 10-y age intervals (the age range was 25–64 y in the 1986 and 1990 surveys and, thereafter, 25–74 y). The present study included survey data from the screenings that were performed in 1986, 1990, 1994, 1999, 2004, 2009, and 2014. The participation rate has varied between 62% and 81% over the years. Health-examination and questionnaire procedures are virtually identical to those in the VIP. Evidence of systematic bias across sociodemographic characteristics over time or between participants and nonparticipants has been minimal (25, 26).

The combined VIP-MONICA data set included 112,519 unique participants (women: 50.8%; $n = 57,160$) with ≥1 health examination that included a diet recording. Of these individuals, 9263 participants (8.2%) were excluded from the present analyses on the basis of incomplete food-intake data, extreme (highest and lowest 1%) food intakes (27), extreme energy intakes [lowest 1% and >20.9 MJ (5000 kcal)], and implausible height (<130 or >210 cm) or weight (<35 kg). Overall, 103,256 participants (women: 51.0%; $n = 52,652$) were included in the study of whom 34,677 participants had a 10-y follow-up visit available. The study flowchart for the present study is shown in **Figure 1**.

Ascertainment of mortality

Mortality endpoints until 31 December 2014 were identified by linking the VIP and MONICA databases with the Swedish national cause-of-death registry. The 12-digit Swedish personal identification numbers were used as the linkage variable. After the exclusions that were described previously, the data set included 6892 deaths (women: 41.7%; $n = 2971$) (Figure 1). The mean ± SD follow-up time was 13.7 ± 6.8 y, and the number of person-years at risk was 1,410,233 y.

Dietary assessments

All participants completed a diet and lifestyle questionnaire that included a semiquantitative Northern Sweden food-frequency questionnaire (FFQ) at each screening visit (http://www.biobank.umu.se/biobank/biobank---for-researchers/northern-sweden-diet-database/). For the present study, all MONICA screenings and VIP visits after 1991 were included and, accordingly, VIP follow-up visits were from 2001 and later. Over the study period, the following 2 versions of the FFQ were used: a longer version (84 food items and aggregates) and a shortened version (64–66 food items and aggregates). The longer version, which was used in the VIP until 1996 and in all MONICA screening occasions except the one in 1990, was completed by 41% of the participants, and the shorter version was completed by 59% of the participants. The shorter version, which has been used continuously since 1997 in the VIP, was reduced by deleting some related food items and merging related items. However, the questions

[11] Abbreviations used: FFQ, food-frequency questionnaire; MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; NSHDS, Northern Sweden Health and Disease Study; SNP, single nucleotide polymorphism; VIP, Västerbotten Intervention Program.

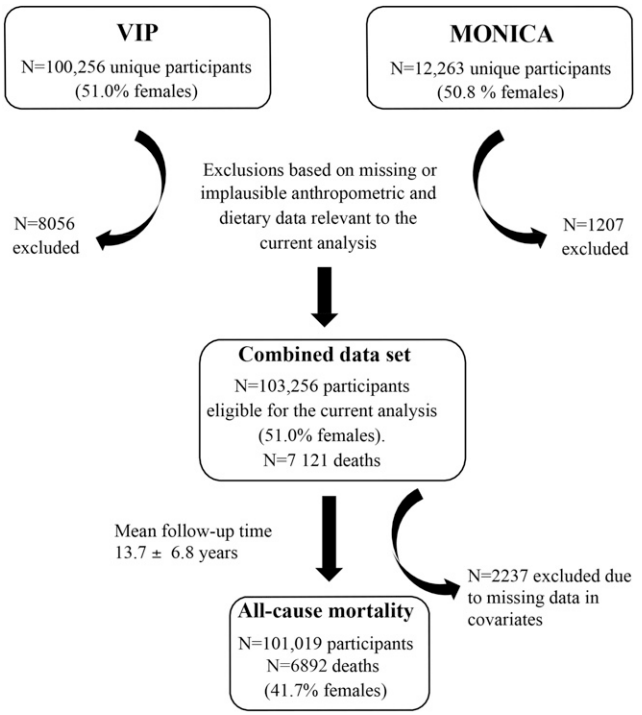

FIGURE 1 Study flowchart illustrating the analysis-specific exclusions that were applied in the VIP and the MONICA study cohorts in setting up the study sample for the current analysis. Intake of dairy products was monitored from 1986 to 2014, and the endpoint for death was 31 December 2014. MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; VIP, Västerbotten Intervention Program.

about nonfermented milk, fermented milk, and butter products have remained identical over time. Before 1991, the FFQ included only one question on hard-cheese intake, and from 1991 and onward, intakes of high- and low-fat types of hard cheeses were queried separately. In the FFQ, intakes were reported on a fixed 9-level scale (28). Meal-time portion sizes were estimated with the support of 4 color pictures of a plate containing increasing amounts of staple foods (potato, rice, and pasta), main protein sources (meat and fish), and vegetables. For other foods, either sex- and age-specific portion sizes or fixed sizes, such as an apple or egg, were applied (28). Total estimated daily intake of energy and nutrients was calculated by weighting reported intake frequencies by the food composition that is provided by the National Food Agency (http://www.livsmedelsverket.se/en/food-and-content/naringsamnen/livsmedelsdatabasen/). Estimated intakes of energy, nutrients, vitamins, and minerals have been validated against repeated 24-h dietary records and biological markers (28–31).

A diet score that reflected healthy eating habits was calculated as previously described (32). Briefly, the daily intake frequency was calculated for 8 food and beverage groups. Favorable food groups included fish, fruit (except juices), vegetables (except potatoes), and whole-grain foods. Unfavorable food and beverage groups included red or processed meats, desserts and sweets, sugar-sweetened beverages, and fried potatoes. Intake frequencies were ranked by sex and 10-y age groups in ascending quartile ranks for favorable food and beverage groups and in descending quartile ranks for unfavorable food and beverage groups. The sum of all quartile ranks represented the Healthy Diet Score with a minimum of 0 and a maximum of 24 and with higher ranks indicating healthier food and beverage choices.

Assessment of potential confounding factors

Body weight (kilograms) and height (meters) were measured with participants wearing light clothes without shoes, and BMI (in kg/m^2) was calculated (body weight divided by the square of height). Participants were categorized as normal weight (BMI <25), overweight (BMI ≥ 25 to <30), and obese (BMI ≥ 30). Information on smoking, the highest obtained level of education, and physical activity was collected from the questionnaires. Smoking was categorized as never smoking, past smoking (having smoked daily or occasionally), and present smoking (daily or occasional smoker). For education, participants were categorized as having a university education or not. The physical activity level was estimated on the basis of the Cambridge Index of Physical Activity (33). This index is a validated index on the basis of information on occupational and leisure-time physical activity, which categorizes participants as inactive, moderately inactive, moderately active, and active, respectively. For the present analyses, the moderately inactive, moderately active, and active categories were combined, thus categorizing participants as inactive or active.

Genotyping

In a subsample of 7404 men and women, DNA was extracted from peripheral white blood cells and diluted to 4 ng/μL as previously described (34). Genotyping of the SNP *LCT-13910 C/T* (rs4988235) was performed with the use of the Sequenom iPLEX platform (Sequenom Inc.) with an SNP call rate $>95\%$. The SNP adhered to Hardy-Weinberg Equilibrium ($P = 0.003$) after adjustment for the total number of SNPs genotyped (i.e., $0.05 \div 32 = 0.001$).

Statistical analyses

Participants were categorized into 4 groups on the basis of reported intakes of nonfermented milk, fermented milk (including soured milk and yogurt), cheese, and butter, respectively [i.e., those reporting intake *1*) never or <1 time/wk, *2*) 1 time/wk to <1 time/d, *3*) 1 to <2.5 times/d, and *4*) ≥ 2.5 times/d]. Participant characteristics by intake category, including frequencies (percentages) and means (95% CIs), were calculated and adjusted for sex and age. Means of nutrient intakes were further adjusted for total energy intake and BMI. Trends in ordinal categories of dairy product intake (order: <1 time/wk, 1 time/wk to <1 time/d, 1 to <2.5 times/d, and ≥ 2.5 times/d) or milk by fat content (order: 3%, 1.5%, and 0.5% fat) were evaluated with the nonparametric Jonckheere-Terpstra trend test. The stability of reported intake of dairy products over time was evaluated in 34,677 participants with FFQ data available from a 10-y follow-up visit in the VIP.

Associations between each of the dairy exposure variables and all-cause mortality were tested in Cox proportional hazards models after the exclusion of individuals with missing data for BMI (0.5%), education (0.7%), and smoking (1.1%), which resulted in 101,019 participants and 6892 deaths (Figure 1). The models were adjusted for an increasing number of potential confounders as follows: sex and age at recruitment (crude model); plus BMI (normal weight, overweight, and obese); plus screening year;

plus smoking status (never, past, or current smoking); plus education (university compared with nonuniversity); plus total energy intake (kilojoules per day) (adjusted model). Models that excluded subjects with missing data on BMI and education were also tested, as these covariates were significantly associated with the all-cause mortality. Missing values for smoking and physical activity were included as separate dummy variables. In subsequent analyses, the Cox proportional hazards models were tested in the 41,676 participants who reported exclusive intake of one type of nonfermented milk (i.e., high-, medium-, or low-fat milk). For these analyses, participants in the lowest category of consumption (i.e., those who reported intake never or <1 time/wk) were excluded because the majority of them were characterized by a preference for another type of nonfermented milk. Dose-response analyses were performed by comparing HRs across the 4 categories of intake (as previously defined). Exclusions were done as previously described. Proportional hazards model assumptions were confirmed with the use of Schoenfeld's test (35). An effect modification by any of the confounders previously mentioned was tested with the use of Wald's test. Heterogeneity was analyzed with the use of chi-square tests. Sensitivity analyses were performed according to the exclusion of participants *1*) with missing data for physical activity (11.0%), *2*) <35 y of age, *3*) reporting intake frequencies greater than the 99th percentile, *4*) of non-Swedish origin by self-report, *5*) who died during the first 2 y of follow-up in line with commonly applied cutoff definitions (36), and *6*) with health examinations before 1991 (i.e., before the question on cheese intake was split into 2 questions and when the major transition from high- to medium-fat nonfermented milk had taken place). Additional adjustment for dairy product–related variables, such as vitamin D, calcium, and lactose (proxy for galactose), and the Healthy Diet Score were done. Furthermore, in an effort to further reduce potential bias from lifestyle variables, analyses were repeated in a restricted more homogeneous group of never and previous smokers with >9 y of education (compulsory school in Sweden) and overall diet pattern assessed by the Healthy Diet Score >12 (median value for the entire cohort).

A Mann-Whitney *U* test was used to study differences in nonfermented milk intake between individuals with different genotypes of the *LCT-13910 C/T* SNP with the use of a dominant genetic model (TT and TC genotype carriers compared with CC genotype carriers) and a codominant genetic model (TT or TC genotype carriers compared with CC genotype carriers). Logistic regression was used to study association between *LCT-13910 C/T* and mortality with the use of dominant and codominant genetic models including sex and age as covariates. These analyses were restricted to individuals of European descent who reported Sweden as their country of origin (n = 7404). All analyses were based on dairy intake that was reported at the baseline visit. All statistical analyses were performed with the use of SAS version 9.4 software (SAS Institute Inc.) or SPSS version 22 (IBM Analytics Inc.) software.

Ethical considerations

The study protocol and data-handling procedures were approved by the Regional Ethical Review Board of Northern Sweden (registration number: 2013/332/31). All study participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Characteristics of study participants stratified by dairy intake

Characteristics of the study participants stratified by reported intake of nonfermented milk (never or <1 time/wk, 1 time/wk to <1 time/d, 1 to <2.5 times/d, and ≥2.5 times/d) are shown in **Table 1**. Although trends were significant, the mean age (range: 47–48 y) and BMI were similar across the 4 intake categories, whereas the proportions of participants with a university education and who were physical inactive decreased with increasing intake of nonfermented milk. Participants who reported nonfermented milk intake ≥2.5 times/d were most likely to be smokers. In addition, participants who reported nonfermented milk intake ≥2.5 times/d reported slightly higher proportions of energy from saturated and *trans* fatty acids, higher vitamin D and lactose intakes, and lower vitamin C intake than participants did who consumed milk less frequently (**Supplemental Table 1**). Information on the country of origin was available in 73% of the participants, which was mainly due to the fact that this information was not collected in the earliest VIP and MONICA screening years. Of individuals who reported their country of origin, the vast majority were of Swedish origin (94%) with the remaining 6% originating from other European (3.4%) and non-European (2.6%) countries.

Differences in participant characteristics varied in magnitude and direction across reported intakes of fermented milk, cheese, and butter; e.g., high consumers of fermented milk were less likely to be smokers and more likely to have a university education than were low consumers, whereas the opposite trend was seen for high consumers of butter compared with low consumers of butter, and an increasing proportion of participants with university education was observed across categories of cheese intake (Table 1). BMI and physical inactivity decreased across increasing intakes of fermented milk, butter, and cheese (Table 1). The proportion of energy intake from saturated fat increased with higher butter, cheese, and fermented milk intakes (Supplemental Table 1).

A comparison of lifestyle variables between participants who reported exclusive intake of high-, medium-, or low-fat milk is presented in **Supplemental Table 2**. Reported average daily milk intake did not differ between the nonfermented milk-fat types, but in general, participants who reported exclusive intake of high-fat nonfermented milk displayed different levels of some lifestyle-related cardiometabolic risk factors. Specifically, there was a lower proportion of individuals with a university education, and there were more smokers in this group, whereas physical activity and obesity showed the opposite trend.

Associations between reported intakes of dairy products and all-cause mortality

Cox regression analyses between all-cause mortality and intakes of nonfermented milk, fermented milk, cheese, and butter, respectively, were performed. As shown in **Table 2**, models that were adjusted for age and sex (crude model) showed positive associations with all-cause mortality for intake of nonfermented milk and butter, whereas inverse associations were obtained for fermented milk and cheese intakes. The associations for nonfermented milk, cheese, and butter remained significant, although

TABLE 1
Characteristics of study participants by category of reported intakes of various dairy products (n = 103,256)[1]

	Intake category				P-ordinal trend
	<1 time/wk	1 time/wk to <1 time/d	1 to <2.5 times/d	≥2.5 times/d	
Nonfermented milk					
Participants, n (%)	15,788 (15.3)	24,916 (24.1)	34,465 (33.4)	28,087 (27.2)	—
Men/women, %	45/55	50/50	45/55	55/45	<0.001
Age, y	47.4 ± 9.1[2]	46.7 ± 9.5	47.3 ± 9.7	48.0 ± 9.9	<0.001
BMI,[3] kg/m^2	25.8 ± 4.3	26.0 ± 4.2	25.9 ± 4.2	26.0 ± 4.3	0.002
Present smokers,[4] %	21.1	18.4	19.5	24.7	<0.001
University education,[4] %	30.1	30.7	29.5	23.5	<0.001
Physically inactive,[4] %	19.3	18.3	18.0	16.9	<0.001
Fermented milk					
Participants, n (%)	26,419 (25.6)	47,505 (46.0)	27,972 (27.1)	1360 (1.3)	—
Men/women, %	57/43	49/51	43/57	42/58	<0.001
Age, y	46.3 ± 9.6	46.4 ± 9.7	48.0 ± 9.6	46.8 ± 9.4	<0.001
BMI,[3] kg/m^2	26.1 ± 4.3	26.0 ± 4.2	25.7 ± 4.1	25.7 ± 4.4	<0.001
Present smokers,[4] %	25.9	20.7	16.7	19.0	<0.001
University education,[4] %	23.2	27.7	33.6	35.3	<0.001
Physically inactive,[4] %	19.3	17.6	17.5	16.2	<0.001
Butter					
Participants, n (%)	32,907 (31.1)	16,238 (15.7)	17,914 (17.3)	36,197 (35.1)	—
Men/women, %	41/59	52/48	50/50	55/45	<0.001
Age, y	47.2 ± 9.6	46.2 ± 9.5	45.9 ± 9.5	47.2 ± 9.9	<0.001
BMI,[3] kg/m^2	26.2 ± 4.3	26.1 ± 4.2	26.1 ± 4.3	25.5 ± 4.1	<0.001
Present smokers,[4] %	20.6	18.3	20.6	22.5	<0.001
University education,[4] %	27.6	33.9	32.1	24.4	<0.001
Physically inactive,[4] %	18.3	17.9	18.4	17.5	0.008
Cheese					
Participants, n (%)	11,752 (11.4)	43,813 (42.4)	34,512 (33.4)	13,179 (12.8)	—
Men/women, %	52/48	53/47	44/56	46/54	<0.001
Age, y	47.0 ± 9.8	46.6 ± 9.6	47.3 ± 9.7	46.0 ± 9.7	0.322
BMI,[3] kg/m^2	26.3 ± 4.5	26.2 ± 4.3	25.7 ± 4.1	25.3 ± 4.1	<0.001
Present smokers,[4] %	21.9	20.6	21.0	20.8	0.410
University education,[4] %	23.4	27.7	29.4	31.3	<0.001
Physically inactive,[4] %	18.2	18.3	17.9	17.0	0.005

[1] Ordinal left-to-right differences between categories were calculated with the use of a nonparametric Jonckheere-Terpstra trend test.
[2] Mean ± SD (all such values).
[3] Standardized for sex and age.
[4] Proportion (percentage) in each intake category.

slightly attenuated, after additional adjustments for BMI, screening year, smoking, education, and total energy intake (adjusted model). Additional adjustment for physical activity did not change overall associations. The inverse association between intake of fermented milk and all-cause mortality disappeared when additionally adjusting for potential confounding lifestyle factors (Table 2). The results were similar (P-heterogeneity > 0.05) in men and women, but in men, the associations of nonfermented and fermented milk with all-cause mortality were no longer significant in the adjusted model, and the same relation was true for fermented milk and butter in women (data not shown).

Association of nonfermented milk with all-cause mortality stratified by fat content

We compared the HRs for all-cause mortality for the 3 types of nonfermented milk that were available on the market [i.e., high-fat milk (3%), medium-fat milk (1.5%), and low-fat milk (0.5%)] in separate models. As shown in **Table 3**, intakes of all 3 types of nonfermented milk showed significant, positive associations with all-cause mortality, and similar results were obtained when the analyses were restricted to participants who reported exclusive intake of one nonfermented milk type. However, the models that included all participants showed slightly higher HRs in subjects who reported intake of high-fat milk than in participants who consumed medium- or low-fat milk, whereas the HRs were more similar in the models that were restricted to exclusive consumers. Overall, adjustment of the models for putative confounding factors slightly reduced the magnitude and significance of the results.

HRs were also compared in exclusive nonfermented milk–type consumers in a Cox proportional model that was based on the fat content with high-fat milk as reference. As shown in **Table 4**, compared with high-fat nonfermented milk, significantly lower HRs were observed for both medium-fat milk and low-fat milk, although the HRs were attenuated in the adjusted models and were nonsignificant for low-fat milk. In line with this, all-cause mortality was significantly higher in exclusive high-fat–milk consumers (11.5%) than in participants who exclusively reported

TABLE 2
HRs (95% CIs) for intakes of dairy products and all-cause mortality calculated from Cox proportional hazard models adjusted for potential confounders[1]

	HR by dairy product			
	Nonfermented milk	Fermented milk	Cheese	Butter
Subjects in analyses,[2] *n*	101,019	101,019	101,019	101,019
Mortality cases, *n*	6892	6892	6892	6892
Person-years, *n*	1,377,035	1,377,035	1,377,035	1,377,035
HR (95% CI)[3]				
Crude model	1.05 (1.03, 1.07)***	0.90 (0.86, 0.94)***	0.93 (0.91, 0.96)***	1.03 (1.01, 1.05)***
Adjusted model	1.02 (1.00, 1.05)*	0.96 (0.92, 1.01)	0.94 (0.91, 0.97)***	1.02 (1.00, 1.04)*

[1] Crude models were adjusted for age and sex, adjusted models were further adjusted for BMI, screening year, smoking, education, and energy intake, and participants with missing values for these covariates were excluded in all models. *$P < 0.05$, ***$P < 0.001$.

[2] Exclusion of participants with missing values for covariates in the adjusted model reduced the number of observations from 103,256 to 101,019 and the number of deaths from 7121 to 6892. Additional adjustment for physical activity (excluding those with missing information) did not affect HRs but reduced the number of subjects in the analyses by another 11,187 persons because Cambridge Index of Physical Activity questions were not included in the initial version of the screening questionnaire.

[3] Energy and dairy intakes were included as reported intakes per day as continuous variables.

intake of medium-fat–milk (6.3%) or low-fat–milk (8.7%) alternatives ($P < 0.001$; chi-square test).

Dose-response analyses

To further explore a dose-response association between intake of nonfermented milk and all-cause mortality, HRs were calculated for increasing categories of intake with adjustment for potential confounders. The reference category was set to participants who reported intake <1 time/d when total nonfermented milk intake was the exposure and 1 time/wk to 1 time/d when the analysis was stratified by the fat content of nonfermented milk. These analyses revealed significant, positive dose-response associations between nonfermented milk intake and mortality (both total intake and by fat content). High consumers of nonfermented milk (≥2.5 times/d) had a 32% increased hazard (HR: 1.32; 95% CI: 1.18, 1.48) for all-cause mortality compared with that of individuals who consumed milk ≤1 time/wk (**Table 5**). The corresponding value for butter was 11% (HR: 1.11; 95% CI: 1.07, 1.21).

Mendelian randomization based on lactase-persistence–associated gene

To investigate if the relation between nonfermented milk intake and all-cause mortality was causal rather than driven by confounding factors, the association between all-cause mortality

TABLE 3
HRs (95% CIs) for nonfermented milk by fat content and all-cause mortality in all participants and consumers reporting intake of one milk type exclusively calculated from Cox proportional hazard models adjusted for potential confounders[1]

	HR by milk fat content		
	High fat (3%)	Medium fat (1.5%)	Low fat (0.5%)
All subjects			
Participants (reported intake ≥1 time/wk),[2] *n*	16,183	62,856	24,699
Mortality cases, *n* (%)	1551 (9.6)	3875 (6.2)	1829 (7.4)
HR (95% CI)[3]			
Crude model	1.13 (1.08, 1.18)***	1.05 (1.01, 1.08)**	1.05 (1.01, 1.10)*
Adjusted model	1.08 (1.03, 1.14)**	1.01 (0.98, 1.05)	1.03 (0.98, 1.08)
Subjects with exclusive milk-type preference[2]			
Participants (reported intake ≥1 time/wk), *n*	6177	27,966	6566
Mortality cases, *n* (%)[2]	710 (11.5)	1769 (6.3)	569 (8.7)
HR (95% CI)[3]			
Crude model	1.12 (1.05, 1.19)***	1.13 (1.08, 1.19)***	1.09 (1.01, 1.18)*
Adjusted model	1.06 (0.99, 1.13)	1.08 (1.03, 1.14)**	1.07 (0.98, 1.16)

[1] Crude models were adjusted for age and sex and adjusted models were further adjusted for BMI, screening year, smoking, education, and energy intake. Participants with missing values for these covariates were excluded in all models. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

[2] Additional adjustment for physical activity with exclusions made for missing information did not affect any HR. Models including all subjects and missing values as a dummy category only affected the HR (95% CI) by 1 unit of the second decimal.

[3] Energy and dairy intakes were included as reported intakes per day as continuous variables.

TABLE 4
Relative associations of nonfermented milk intake by fat content and all-cause mortality in consumers reporting intake of one milk type exclusively calculated from Cox proportional hazard models adjusted for potential confounders[1]

	HR between milk type[2]			
	High fat (3%)	Medium fat (1.5%)	Low fat (0.5%)	*P*-trend
Participants (reporting intake ≥1 time/wk), *n*	6177	27,966	6566	—
Mortality cases, *n* (%)	710 (11.5)	1769 (6.3)	569 (8.7)	—
HR (95% CI)[2]				
Crude model	Reference	0.78 (0.71, 0.85)***	0.84 (0.75, 0.94)**	0.002
Adjusted model	Reference	0.90 (0.82, 0.98)*	0.94 (0.84, 1.05)	0.101

[1] Crude model was adjusted for age and sex, and the adjusted model was further adjusted for BMI, screening year, smoking, education, and energy intake. Participants with missing values for these covariates were excluded in all models. *P*-trend values were calculated by treating categories of milk as a continuous variable (i.e., high fat = 1, medium fat = 2, and low fat = 3). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

[2] Milk type was included in the model as a categorical variable, and high-fat nonfermented milk intake is the reference. The analyses were restricted to consumers reporting exclusive intake of high-, medium-, or low-fat nonfermented milk ≥1 time/wk. Energy was included as reported intake per day as a continuous variable. Additional adjustment for physical activity with exclusions made for missing information did not affect any HR. Models including all subjects and missing values as dummy categories only affected the HR and 95% CI by 1 unit of the second decimal.

and the *LCT-13910 C/T* variant was tested. Of 7404 participants of European origin (who reported Sweden as their country of origin) with available genotype data, 484 individuals (6.5%) had the CC lactase-nonpersistent genotype, whereas 2630 individuals (35.5%) and 4290 individuals (58.0%) had either TC or TT lactase-persistent genotypes, respectively.

Nonfermented milk intake was significantly lower in participants with the CC genotype than in participants with the TC genotype ($P = 2.91 \times 10^{-5}$; Mann–Whitney *U* test) or TT genotype ($P = 1.21 \times 10^{-6}$; Mann–Whitney *U* test) under a co-dominant genetic model (**Table 6**). Similar results were observed for the CC-genotype group compared with the TC- and TT-genotype groups combined under a dominant genetic model ($P = 2.14 \times 10^{-6}$; Mann–Whitney *U* test). The numbers of study participants by genotype and nonfermented milk–intake category are presented in Table 6.

Compared with CC lactase-nonpersistent participants, mortality ORs in a co-dominant model were 1.08 (95% CI: 0.80, 1.46) for TC lactase-persistent participants and 1.06 (95% CI: 0.79, 1.43) for TT lactase-persistent participants. For the dominant model, the mortality OR was 1.07 (95% CI: 0.80, 1.43) for TC and TT lactase-persistent participants compared with CC lactase-nonpersistent participants (Table 6).

Stability of intakes from baseline to follow-up

Of 103,256 participants, 34,677 VIP participants with a baseline visit in 1991 or later had a 10-y follow-up and were administered a second FFQ that allowed for an evaluation of the stability of dairy intake over time. Overall, the proportion of participants who reported intakes of various dairy products including nonfermented milk in total and by fat content were similar over the 10-y period, and similar results were observed for participants who reported exclusive intake of 1 type of nonfermented milk by fat content at baseline ($n = 13,856$ persons) (**Supplemental Table 3**).

Sensitivity analyses

Several sensitivity analyses were performed including additional adjustment for a Healthy Diet Score and intakes of lactose (also a proxy for galactose), vitamin D, and calcium, respectively. Separate analyses were also performed with the exclusion of participants who *1*) had an incomplete set of physical activity questions for the Cambridge Index of Physical Activity, *2*) were <35 y of age, *3*) were characterized by extreme dairy intakes ($n = 878$), *4*) reported a non-Swedish origin, *5*) had died during the first 2 y of follow-up ($n = 9834$), or *6*) were recruited before 1991 (before the major transition from high-fat to medium-fat nonfermented milk occurred). None of the sensitivity analyses materially changed the results. Finally, none of the covariates included in our models showed evidence of acting as an effect modifier of the association between each dairy exposure and all-cause mortality.

Overall, the HRs (95% CIs) for intakes of various dairy products and all-cause mortality in the restricted more homogeneous sample ($n = 33,486$) (**Supplemental Table 4**) were in line with those seen in all participants (Table 2), although significance was no longer reached. Thus, for nonfermented milk, the HR in the restricted group was 1.04 (95% CI: 0.98, 1.11; $P = 0.186$).

DISCUSSION

The present study investigated the association between intake of dairy products and all-cause mortality in adults within the VIP and MONICA study, both of which are population-based cohorts from Northern Sweden and parts of the NSHDS. The Northern Sweden population is characterized by high nonfermented milk consumption and lactase persistence. Thus, average intake of nonfermented and fermented milk in Swedes has been estimated to be 83 kg/y (95th percentile: 195 kg/y) of which 50 kg/y (95th percentile: 83 kg/y) constituted nonfermented milk (37). We were able to confirm the positive association between nonfermented milk intake and all-cause mortality that was previously reported by Michaëlsson et al. (19) in another Swedish population and further extended this finding to show that the association with all-cause mortality was present regardless of the fat content of nonfermented milk, albeit it was most pronounced for high-fat nonfermented milk. In addition, butter intake was positively associated with all-cause mortality, whereas intake of fermented dairy products [i.e., cheese and fermented milk (soured

TABLE 5
HRs (95% CIs) by reported intake category of nonfermented milk stratified by fat content and all-cause mortality calculated from Cox proportional hazard models adjusted for potential confounders[1]

		HR (95% CI) with increasing number of potential confounders in the model	
Intake categy[2]	*n*	Crude model	Adjusted model
Total milk			
<1 time/wk	7616	Reference	Reference
1 time/wk to <1 time/d	8729	0.97 (0.86, 1.10)	0.97 (0.86, 1.10)
1 to <2.5 times/d	13,418	1.15 (1.03, 1.29)	1.12 (1.00, 1.25)*
≥2.5 times/d	10,946	1.32 (1.18, 1.48)***	1.18 (1.06, 1.33)**
P-trend		0.052	0.157
High-fat milk			
1 time/wk to <1 time/d	1698	Reference	Reference
1 to <2.5 times/d	1686	1.18 (0.96, 1.45)	1.12 (0.91, 1.39)
≥2.5 times/d	1881	1.28 (1.05, 1.56)*	1.10 (0.89, 1.35)
P-trend		0.011	0.368
Medium-fat milk			
1 time/wk to <1 time/d	8274	Reference	Reference
1 to <2.5 times/d	9478	1.18 (1.04, 1.33)*	1.14 (1.00, 1.29)*
≥2.5 times/d	7200	1.38 (1.21, 1.57)***	1.24 (1.09, 1.42)**
P-trend		<0.001	0.002
Low-fat milk			
1 time/wk to <1 time/d	1647	Reference	Reference
1 to <2.5 times/d	2254	1.19 (0.94, 1.48)	1.16 (0.92, 1.46)
≥2.5 times/d	1865	1.28 (0.99, 1.56)	1.18 (0.93, 1.49)
P-trend		0.041	0.155

[1] Crude models were adjusted for age and sex and adjusted models were further adjusted for BMI, screening year, smoking, education, and energy intake. Participants with missing values for these covariates were excluded in all models. *P*-trend values were calculated by treating categories of milk intake as continuous measures. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

[2] For total milk, all 4 categories of intake (compare with Table 1) were included, and for the nonfermented milk by fat content, the analysis was restricted to participants reporting intake ≥1 time/wk with the exclusive consumption of 1 milk type. Energy intake was included as reported intake per day as continuous variables. Additional adjustment for physical activity with exclusions made for missing information did not affect any HR. Models including all subjects and missing values as a dummy category affected the HRs (95% CI) by only 1 unit of the second decimal.

milk and yogurt)] were associated with lower risk of all-cause mortality in this study. Therefore, our results support a difference in the relation between nonfermented dairy products (regular milk and butter) and fermented dairy products (fermented milk and cheese) and all-cause mortality. Although we were not able to test soured milk and yogurt separately, similar traits and fermentation processes with added bacteria, combined with soured milk being the commonly consumed product in the study region, argue for similar biological effects of soured milk and yogurt. Calcium, vitamin D, and lactose intakes could not explain the observed associations between dairy exposures and all-cause mortality.

Studies that have investigated dairy intake as a primary or secondary exposure in relation to all-cause mortality have been either conflicting (5, 6, 19, 38, 39) or did not study the effects of single dairy foods (4, 9, 40). Therefore, we believe that our results contribute to the understanding of the different associations of single dairy-food products and health. Intake of nonfermented milk and a preference for high-fat–containing nonfermented milk were associated with a lower educational level, whereas the opposite association was true for cheese. We were not able to exclude residual confounding by socioeconomic or lifestyle factors, which was further supported by all HRs being attenuated after adjustment for the potential confounding lifestyle factors. It is difficult to draw conclusions from observational studies as to whether identified associations are causal or due to confounding by other related factors, which calls for less bias-sensitive methods or intervention studies. Because genotypes are assigned randomly at conception, the results obtained from analyses including genetic variants are not prone to confounding by lifestyle factors.

Here, we used the *LCT-13910 C/T* SNP in the framework of Mendelian randomization and showed mortality ORs pointing toward a potential causal association. However, the 95% CIs were wide and included zero, which indicated that we did not have sufficient statistical power in these analyses to accept or reject the support for a causal association. Therefore, the Mendelian randomization analyses should be repeated in a considerably larger study sample, and the presented results might be an incentive for a larger meta-analysis effort.

To our knowledge, this is the second study that shows a positive association between nonfermented milk intake and all-cause mortality in a Swedish population (19). As previously stated, dairy intakes in Sweden are among the highest worldwide (11) and are dominated by milk, with an average of 60% of milk being nonfermented (37). Thus, the positive association between intake of nonfermented milk and mortality might only be observable in countries with such a high total and wide variation in exposure as in Sweden. This may, at least in part, explain some of the discrepancies between previous studies.

There are several plausible biological explanations that could explain the opposite results for fermented and nonfermented dairy products. One possible explanation involves dairy fatty acids. However, our results do not support a simple detrimental effect of dairy fat. Although butter intake was positively associated with all-cause mortality, hard cheese, which is dominated by high-fat alternatives (>28% weight) in Sweden, was an inverse predictor. Indeed, cheese has been reported to increase LDL-cholesterol concentrations less strongly than butter at an equal fat content, thereby suggesting potential beneficial effects of cheese (including bacterial fermentation and proteolysis during ageing) over butter (41). The production of cheese curd from nonfermented milk is essentially a process in which both fat and casein are concentrated ∼10-fold, whereas whey proteins, lactose, and soluble salts and enzymes are removed (42). Therefore, a possible hypothesis to explain the opposite associations of nonfermented milk compared with cheese is that production and bacterial fermentation reduce the galactose content in hard cheese. A detrimental effect of galactose is a hypothesis that has been suggested and seemingly supported by Michaëlsson et al. (19). However, the fact that adjustment for lactose intake (as a proxy for galactose intake) did not attenuate our observed associations suggests that other biological explanations may be involved. A second potential mechanism relates to the presence of bioactive peptides and host-receptor mimicking epitopes in fermented dairy products that can influence the gut microbe–host interaction with subsequent effects on immune function, cell signaling,

TABLE 6
Intake frequency of nonfermented milk and mortality ORs by *LCT-13910* genotypes (rs4988235) (n = 7404)[1]

Genotype	Intake category of nonfermented milk				P[2]				OR (95% CI) [P][2]	
	<1 time/wk	1 time/wk to <1 time/d	1 to <2.5 times/d	≥2.5 times/d	Mann-Whitney, codominant model	Mann-Whitney, dominant model	Total, n	Dead, n (%)	Codominant model	Dominant model
CC (lactase nonpersistent)	114	120	160	90	Reference	Reference	484	60 (12.4)	Reference	Reference
TC (lactase persistent)	435	602	939	654	2.9×10^{-5}	2.1×10^{-6}	2630	352 (13.4)	1.08 (0.80, 1.46) [0.621]	1.07 (0.80, 1.43) [0.651]
TT (lactase persistent)	670	968	1544	1108	1.2×10^{-6}		4290	581 (13.5)	1.06 (0.79, 1.43) [0.685]	

[1] Analyses were restricted to individuals of European descent (reporting Sweden as their country of origin) per self-report.
[2] Models were adjusted for age and sex.

and overall bacteria attachment and colonization (43, 44). A different stimulation of gut-bacteria activity after intake of either milk or cheese was suggested in a recent metabolomics study that showed that the microbiota-related metabolite hippurate was significantly higher in participants who ate cheese than in milk consumers and control participants (45). In the same study, cheese consumption was associated with an increased concentration of short-chain fatty acids in the gut, which was possibly induced by gut-microbiota modulation. Finally, it should be underscored that both lifestyle and causal factors are likely to interact in an individual-determined fashion.

The strengths of the present study are the large sample size, the possibility to stratify the analyses of nonfermented milk by fat content, and the possibility to adjust for several potential confounders. Nonetheless, there are weaknesses that need to be taken into account. We are not able to exclude potential residual confounding by lifestyle variables, we are not able to adjust for galactose exposure directly but use lactose intake as a proxy, and our Mendelian randomization analyses are underpowered. Finally, although we confirm that rankings of dairy product intake are similar for a large portion of the participants with 10-y follow-up recordings, we believe that the analyses in participants with repeated measures are not justified. This is because the reduced number of observations, compared with the main analyses, would have caused a reduction in statistical power and a lack of information.

In conclusion, the positive association between total nonfermented milk intake and all-cause mortality can now be confirmed, and fermentation is likely to counteract the association of milk with all-cause mortality in the current study. Furthermore, all nonfermented milk fat types are independently associated with increased HRs, but compared with full-fat milk, the HRs are lower in consumers of medium- and low-fat milk. However, the question of whether the observed associations are due to confounding lifestyle factors cannot be answered by our Mendelian randomization substudy and needs to be further explored in larger samples.

The authors' responsibilities were as follows—GT, LMN, DS, FR, AW, and IJ: designed the study; FR, IJ, and LL: provided essential materials; GT, LMN, DS, and IJ: analyzed the data; GT and IJ: wrote the manuscript and had primary responsibility for the final content of the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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Non-fermented milk and other dairy products: associations with all-cause mortality

For on-line publication

Supplemental Table 1. Characteristics of study participants diet intake by category of reported intake of various dairy products (N=103 256). Values are means (SD) standardized for reported energy intake, sex, age and BMI. Numbers in each group are given in Table 1. Ordinal left to right differences between categories were calculated by a non-parametric Jonckheere-Terpstra trend test.

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Supplemental Table 3. Associations between milk intakes reported 10-years apart. Repeated measures were available for 34 677 participants who participated in the VIP study from 1991 and onwards.

Supplemental Table 4. Hazard ratios (95% CI) for intakes of dairy products and all-cause mortality calculated from Cox-Proportional Hazard models adjusted for potential confounders in subjects who were never or previous smokers, with >9 years of education and with a diet score ≥12.

Supplemental Table 1. Characteristics of study participants diet intake by category of reported intake of various dairy products (N=103 256). Values are means (SD) standardized for reported energy intake, sex, age and BMI. Numbers in each group are given in Table 1. Ordinal left to right differences between categories were calculated by a non-parametric Jonckheere-Terpstra trend test.

	Intake categories[1]				Ordinal trend P-value
	<1/week	1/week to <1/day	1 to <2.5/day	≥2.5/day	
Non-fermented milk					
Total fat, E%	35.6 (7.7)	35.6 (6.9)	34.6 (6.5)	34.6 (6.4)	<0.001
Saturated fat, E%	14.2 (3.9)	14.6 (3.6)	14.5 (3.4)	15.2 (3.6)	<0.001
Transfat, E%	0.92 (0.52)	0.94 (0.49)	0.97 (0.49)	1.03 (0.51)	<0.001
Wholegrain, g/9 MJ	81.9 (37.9)	83.0 (34.9)	86.1 (34.5)	81.6 (33.0)	0.043
Vitamin C, mg/9 MJ	106.1 (60.0)	96.7 (49.2)	95.6 (49.1)	83.6 (43.3)	<0.001
Vitamin D, µg/9 MJ	6.2 (1.9)	6.4 (1.7)	6.5 (1.6)	7.2 (1.7)	<0.001
Lactose, g/9 MJ	7.8 (6.5)	13.9 (6.2)	20.0 (6.7)	34.5 (9.6)	<0.001
Healthy Diet Score[2]	12.1 (4.1)	11.9 (3.9)	12.0 (4.0)	11.3 (4.0)	<0.001
Fermented milk					
Total fat, E%	36.1 (7.4)	35.1 (6.5)	33.8 (6.3)	33.3 (7.2)	<0.001
Saturated fat, E%	14.9 (4.0)	14.6 (3.4)	14.5 (3.4)	14.9 (4.1)	<0.001
Transfat, E%	1.00 (0.56)	0.97 (0.49)	0.94 (0.45)	0.94 (0.44)	<0.001
Wholegrain, g/9 MJ	72.7 (39.6)	77.7 (36.6)	82.0 (35.5)	80.7 (41.0)	<0.001
Vitamin C, mg/9 MJ	85.7 (56.9)	87.7 (52.6)	88.8 (52.9)	83.1 (53.7)	<0.001
Vitamin D, µg/9 MJ	6.3 (2.0)	6.2 (1.8)	5.9 (1.8)	5.9 (2.2)	<0.001
Lactose, g/9 MJ	15.0 (13.1)	18.4 (11.4)	23.4 (10.7)	34.8 (12.6)	<0.001
Healthy Diet Score[2]	10.9 (4.1)	11.7 (3.9)	12.8 (4.0)	13.3 (4.2)	<0.001
Butter					
Total fat, E%	32.1 (6.1)	32.9 (5.9)	35.6 (6.3)	38.2 (6.4)	<0.001
Saturated fat, E%	12.5 (2.6)	13.4 (2.6)	15.2 (3.1)	17.0 (3.4)	<0.001
Transfat, E%	0.96 (0.61)	0.85 (0.48)	0.88 (0.41)	1.08 (0.41)	<0.001
Wholegrain, g/9 MJ	82.1 (40.0)	75.8 (37.1)	71.1 (35.0)	77.8 (35.5)	<0.001
Vitamin C, mg/9 MJ	95.7 (59.3)	92.4 (55.8)	87.5 (52.9)	77.6 (45.6)	<0.001
Vitamin D, µg/9 MJ	6.3 (1.9)	6.0 (2.0)	5.9 (1.9)	6.2 (1.8)	<0.001
Lactose, g/9 MJ	19.6 (12.9)	19.4 (12.6)	19.1 (12.4)	18.5 (11.6)	<0.001
Healthy Diet Score[2]	12.2 (4.1)	12.0 (4.0)	11.7 (4.0)	11.4 (4.0)	<0.001
Cheese					
Total fat, E%	34.3 (7.8)	34.8 (6.9)	35.0 (6.4)	36.1 (6.4)	<0.001
Saturated fat, E%	13.8 (4.1)	14.3 (3.6)	14.8 (3.3)	16.0 (3.5)	<0.001
Transfat, E%	0.85 (0.53)	0.91 (0.48)	1.03 (0.49)	1.13 (0.51)	<0.001
Wholegrain, g/9 MJ	75.7 (42.2)	75.4 (36.5)	79.8 (36.6)	81.3 (37.0)	<0.001
Vitamin C, mg/9 MJ	90.2 (61.0)	87.4 (53.8)	88.4 (52.6)	83.0 (50.1)	0.045
Vitamin D, µg/9 MJ	6.3 (2.2)	6.3 (1.9)	6.0 (1.7)	5.7 (1.7)	<0.001
Lactose, g/9 MJ	20.0 (14.8)	19.9 (12.5)	18.6 (11.5)	17.1 (10.9)	<0.001
Healthy Diet Score[2]	11.7 (4.3)	11.7 (4.0)	11.9 (4.0)	12.0 (4.1)	<0.001

[1]MONICA participants screened in 1990 were not included due to different FFQ.

[2]Underlying ranking was in sex and age strata.

Supplemental Table 2. Characteristics of participants who reported exclusive consumption of one type of milk, i.e. high (3% fat), medium (1.5% fat) or low (0.5% fat) fat milk only. Values are means (SD) unless otherwise indicated. Ordinal left to right differences between groups were calculated by a non-parametric Jonckheere-Terpstra trend test.

	High fat (3%) milk exclusively	Medium fat (1.5%) milk exclusively	Low fat (0.5%) milk exclusively	Ordinal trend P-value
Participants, n	6 377	28 575	6 724	
Mortality cases, n (%)	737 (11.6)	1 769 (6.2)	593 (8.8)	
Males/females, %	52/48	45/55	42/58	<0.001
Age, y	47.8 (10.2)	46.5 (9.6)	49.1 (9.4)	<0.001
BMI[1], kg/m^2	25.4 (4.2)	25.8 (4.2)	26.6 (4.5)	<0.001
BMI class, %				
normal	50.2	48.1	39.3	<0.001
over weight	36.7	37.9	41.8	<0.001
obese	13.1	14.0	18.9	<0.001
Smoking, %				
present	28.1	21.4	21.4	0.351
past	27.3	29.5	33.3	<0.001
never	44.6	49.1	45.3	<0.001
University education, %	19.1	26.1	29.2	<0.001
Inactive, %	17.5	17.9	19.4	0.005
Marital status, %				
married	76.5	82.4	82.5	<0.001
divorced	8.2	7.2	6.6	<0.001
single	13.3	8.7	9.2	<0.001
widow/widower	2.0	1.8	1.7	0.29
Swedish origin[2], %	90.7	95.6	93.9	<0.001
Sick leave[3], %	21.1	18.3	23.0	0.003
Milk intake[4], servings/d	1.2 (1.1)	1.2 (1.0)	1.2 (1.0)	0.006
Nutrient intake[5]				
total fat, E%	38.0 (7.2)	35.3 (6.5)	32.1 (6.7)	<0.001
saturated fat, E%	17.1 (4.2)	14.8 (3.3)	12.7 (3.2)	<0.001
transfat, E%	1.16 (0.54)	0.97 (0.49)	0.92 (0.55)	<0.001
wholegrain, g/9 MJ	73.0 (36.4)	78.3 (34.8)	81.2 (37.9)	<0.001
vitamin C, mg/9 MJ	83.2 (49.0)	87.4 (49.6)	100.5 (59.0)	<0.001
vitamin D, µg/9 MJ	5.0 (1.8)	6.2 (1.7)	6.5 (1.8)	<0.001
lactose, g/9 MJ	17.1 (10.9)	18.4 (10.9)	20.1 (12.2)	<0.001
Healthy diet score[6]	10.6 (4.1)	11.7 (3.9)	12.8 (4.2)	<0.001

[1] Standardized for sex and age; [2] Information available in 75,686 participants; [3] Continuous for 6 months or longer; [4] Adjusted for sex, age and BMI; [5] Energy standardized intake adjusted for sex, age and BMI. MONICA screened in 1990 were not included; [6] Underlying ranking was in sex and age strata.

Supplemental Table 3. Associations between milk intakes reported 10-years apart. Repeated measures were available for 34 677 participants who participated in the VIP study from 1991 and onward and 10-year follow-ups from 2001 and onward. Data are percent (%) of participants with intake ≥1/week of all subjects (upper panel) or subjects with and exclusive milk preference (lower panel), respectively.

	Non-fermented milk	Fermented milk	Cheese	Butter	High fat milk (3%)	Medium fat milk (1.5%)	Low fat milk (0.5%)
All subjects (n=34 677) [1]							
Proportion with intake ≥1/week							
at baseline	89	79	93	65	15	65	24
10-years later	85	79	91	70	12	61	27
Quintile classification							
correct lowest quintile	52.1	46.5	39.8	50	75.0	43.7	69.3
correct highest quintile	45.4	39.4	39.7	51	43.3	34.2	49.4
Subjects with an exclusive milk preference (n=13 856)							
Proportion with intake ≥1/week							
at baseline	−		−	−	11	65	14
10-years later	−		−	−	10	60	23
Quintile classification							
correct lowest quintile	−		−	−	75.7	42.6	68.6
correct highest quintile	−		−	−	53.4	32.7	53.4

Supplemental Table 4. Hazard ratios (95% CI) for intakes of dairy products and all-cause mortality calculated from Cox-Proportional Hazard models adjusted for potential confounders in subjects who were never or previous smokers, with >9 years of education (compulsory school in Sweden), and with a diet score ≥12 (corresponding to the median value among all 103,256 participants). Crude models were adjusted for age and sex and adjusted models also for BMI, screening year, smoking, education, and energy intake, and participants with missing values for these covariates were excluded in all models. ns for p≥0.05, * for p<0.05, ** for p<0.01, and *** for p<001.

	Hazard ratios by dairy product			
	Non-fermented milk	Fermented milk	Cheese	Butter
Subjects in analyses[1], N	33 486	33 486	33 486	33 486
Mortality cases	1 135	1 135	1 135	1 135
Person-years	420 054	420 054	420 054	420 054
Hazard ratios (95% CI)[2]				
crude model	1.05 (1.00, 1.12)^{ns}	1.00 (0.91, 1.12)^{ns}	0.95 (0.89, 1.01)^{ns}	1.02 (0.97, 1.06)^{ns}
adjusted model[2]	1.04 (0.98, 1.11)^{ns}	1.00 (0.90, 1.11)^{ns}	0.92 (0.86, 0.99)*	1.01 (0.97, 1.06)^{ns}

[1]The study group was restricted to individuals with a relatively higher education level and healthy lifestyle in an attempt to minimize confounding.

[2] Energy and dairy intake were included as reported intake per day as continuous variables.

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ORIGINAL CONTRIBUTION

Dairy product intake and mortality in a cohort of 70-year-old Swedes: a contribution to the Nordic diet discussion

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Abstract
Introduction Conflicting results in the literature exist on the role of dairy products in the context of a Nordic Healthy Diet (NHD). Two recent Swedish studies indicate both negative and positive associations with total mortality when comparing key dairy products. There is no consensus about how to include these foods into the NHD.
Purpose To study consumption of cheese and milk products (milk, sour milk and unsweetened yoghurt) by 70-year-old Swedes in relation to all-cause mortality.
Methods Cox proportional hazard models, adjusted for potential confounders and stratified by follow-up duration, were used to assess the prediction of all-cause mortality by the above foods. The associations of fat from cheese and milk products with mortality were tested in separate models.
Results Cheese intake inversely predicted total mortality, particularly at high protein intakes, and this association decreased in strength with increasing follow-up time. Milk products predicted increased mortality with stable HRs over follow-up. The association between milk products and mortality was strongly influenced by the group with the highest consumption. Fat from cheese mirrored the protective association of cheese intake with mortality, whereas fat from milk products predicted excess mortality, but only in an energy-adjusted model.
Conclusion Based on our results, it may be argued that the role of dairy products in the context of a Nordic healthy diet should be more clearly defined by disaggregating cheese and milk products and not necessarily focusing on dairy fat content. Future epidemiological research should consider dairy products as disaggregated food items due to their great diversity in health properties.

Keywords Elderly · Aged · Nordic diet · Diet quality · Dairy products · Cheese · Mortality

Introduction

Nordic nutrition researchers have recently defined a new healthy diet, which could possess the same health properties as of the well-known Mediterranean diet, but based on typical Nordic foods that are frequently consumed by the Nordic population [1]. The positive health effects of this diet have been attributed to foods that can be locally produced in the Nordic countries (e.g. apples, pears and berries, root vegetables and cabbages, wholegrain oats and rye, salmon and herring as well as boiled potatoes), thereby excluding typical Nordic foods with potential harmful effects such as fresh and processed meat. Notably, dairy products, which were usually considered unhealthy in the Mediterranean diet score [2, 3], have often been excluded from the definition of a healthy Nordic diet [4–6], or considered only if low in fat [7–9], in line with the Nordic Dietary Recommendations [1]. The ambiguous role of dairy products in the definition of the healthy Nordic diet may relate to the fact that their health properties have long been a sensitive topic in Sweden

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and elsewhere. Indeed, the promotion of the health properties of dairy products has a long history in Sweden; a milk propaganda campaign many decades ago was particularly successful in making Swedes become among the greatest per capita milk consumers worldwide [10].

Recent evidence from two large population studies in Sweden re-opened the discussion about the health effects of dairy products by demonstrating that higher milk intakes were positively associated with all-cause mortality, whereas cheese appeared to be protective [11, 12]. However, a recent meta-analysis summarizing the evidence about the association between intake of milk and milk products on mortality showed an overall neutral effect of dairy products [13].

Considering the above premises, and based on prospective population studies of men and women living in Gothenburg [14–19] who underwent diet history interviews at the age of 70, our goal was to determine whether cheese intake was associated with mortality in a different way, compared to milk products.

Subjects and methods

Study population

This study is based on the Gerontological and Geriatric Population Studies in Gothenburg (H70) a prospective cohort study that recruited 1381 men and women aged 70 and born in 1901, 1911, 1922 and 1930 [14, 15, 17–20]. Women belonging to the two latest-born cohorts were jointly examined with the Prospective Population Study of Women in Gothenburg (PPSW) [16]. In both studies, subjects were sampled based on the day of birth, and invited to participate. Response rate decreased with time, from 84 and 86% in the earliest birth cohort to 65% in the most recent birth cohorts with general similar rates in men and women in most cohorts.

These cohorts have been monitored continuously for mortality by linking personal identification numbers with the national death registration system. The present study is based on the mortality follow-up on May 21, 2010 with a mean follow-up of 13.2 years (max follow-up duration = 43 years).

For subjects with incomplete information on key covariates, decisions on exclusions were based on analyses of mortality (not shown). Subjects with missing BMI ($n = 165$) were excluded because of increased mortality risk compared to rest of the population. Since this was not the case for subjects with missing education ($n = 39$) and physical activity ($n = 13$), dummy variables were added to the models to retain these subjects into the analyses. For subjects with missing marital status ($n = 3$) a dummy variable could not be created because of the small number and these subjects were therefore not retained in fully adjusted models. The final study sample included 1213 subjects (678 females) of whom 833 died of any cause during the observation period (431 females).

Dietary assessment

All subjects in the prevent analysis had diet history data (described below) from 1971, 1981, 1992 or 2000. This method, which has been extensively described elsewhere, provided a detailed assessment of usual intakes of a large number of food items and mixed dishes [14, 17–19]. The dietary assessment was previously validated by comparison of energy intake with estimated total energy expenditure (TEE) by heart rate monitoring, activity diary and double labelled water as well as by calculating the ratio between energy intake and basal metabolic rate (BMR) [17, 19, 21]. The dietary assessment consisted of individual interviews by trained dieticians, who enquired about the intakes of food items through a multi-pass process that ensured a high level of completeness. Therefore missing values were reasonably interpreted as null intakes and set to 0 g/day.

Two dietary variables were generated from the sum of either cheese intakes (all types) or a group of dairy products including milk, soured milk and unsweetened yoghurt, hereby referred as "milk products" for simplicity. Although the latter items appeared in all surveys, they could not be analyzed separately since they were combined differently in the various cohorts, but we were able to harmonize them into a single food group for this paper. Fat intakes from both food groups were also calculated. Total protein intake was divided by body weight and dichotomized based on a cut-off of 1.2 g/kg body weight/day [22].

Covariates measured at baseline

Body weight and height were measured by trained staff on the day of the physical examination as described previously [15, 23] and BMI (weight/height^2) was calculated. Physical activity was assessed by asking subjects about their leisure time physical activity during adult life and levels were dichotomized into "physically active" and "physically inactive" [23]. Information about smoking history, education, and current marital status were obtained for all subjects.

Statistical analyses

Continuous variables representing either cheese or milk product intakes were tested in relation to their associations with all-cause mortality in Cox proportional hazard models. In the latter, the follow-up time was included as number of days after the baseline examination. These models were either adjusted for sex and birth cohort only or adjusted for

the following covariates: birth cohort, sex, marital status (married/not married), BMI, smoking status (ever vs never smokers), education (basic vs higher levels) and physical activity level (low activity vs higher). In complementary analyses, high protein intakes (> 1.2 g/kg body weight), alcohol intake and fat intake were separately included in the models.

Since assessment of the risk associated with an increased intake of 1 g per day would have generated very small HRs, intakes were divided by either 10 (cheese) or 100 (milk products) in order to increase the interpretability of hazard ratios. In terms of portions, 10 g correspond to the average single portion of cheese consumed in Sweden, where it is common to cut cheese in thin slices, whereas 100 g of milk products corresponds to approximately 1/5 of a pint, a measure commonly referred to in previous studies [13]. The Cox models were run stratifying by birth cohort by means of the STRATA command available in PROC PHREG (SAS 9.4). This allows the baseline risk for each birth cohort to vary, taking into consideration potential differences in total mortality risk among birth periods without reducing the power of the statistical analyses. Because the assumption of proportional hazards was not fulfilled in this study, we chose to present the results stratified by duration of follow up. Additionally, in order to assess whether the use of dummy variables to retain subjects with missing information regarding potential confounders could have altered our results, we repeated our main analyses using multiple imputation [24] which estimated missing values for education and physical activity. To assess whether the presence of subclinical or pre-existing conditions at the time of the recruitment was likely to have influenced the above-mentioned analyses, we repeated the latter by excluding the subjects who died during the first 2 years of follow-up.

In addition to the analysis of continuous intakes, dose–response was tested by comparing the first sex-specific tertile of cheese and milk product intakes with the second and third tertiles, in Cox models adjusted for the above-mentioned covariates. Also, to further analyze potential non-linear relations between the intakes and mortality, we compared the likelihoods of three different models: (1) those with cheese and milk product intakes included as continuous variables, (2) those also including a quadratic term (i.e. squared cheese intake or squared milk product intakes), and (3) those using piecewise regression models [25] to produce separate HRs on four intervals of intakes. An interaction between cheese and milk products with respect to mortality was tested to determine whether the effect of one dairy product might be modified by the other. A likelihood ratio test comparing model chi square values assessed whether the difference in the overall model chi square (-2Log Likelihood) of two models (e.g. including an extra factor such as milk in the cheese models or an interaction factor between cheese and milk) is significant, assuming that the model with the highest likelihood gives the best prediction of mortality. This test is asymptotically the same as the result reported from the Wald test (where two variables plus their product are included in the same model), but is considered more reliable when the sample size is limited. By applying the same procedure, we tested effect modification by alcohol intake on the association of either cheese or milk product intakes on total mortality. In order to assess whether the participants' nutritional status could have modified the association between dairy intake and mortality, we tested effect modification by BMI and high protein intake (dichotomized using 1.2 g/kg body weight as threshold [22]) on the association of these food groups with mortality.

Fat intakes from either cheese or milk products were tested against mortality in Cox models adjusted for the above-mentioned covariates plus total fat intake. Analyses have been performed in SAS 9.4 with the exception of multiple imputation [24] which has been done in Stata 13.

Bioethics

All examinations since 1992 were approved by the Gothenburg University Ethics Committee. In accordance with the Declaration of Helsinki (1989) of the World Medical Association, all participants were informed of the aims and procedures of the study and gave their consent (Ethical approval no. 179–92 and no. Ö 402–99).

Results

Descriptive analyses

Covariates included in the analyses are described after stratification by birth cohort (Table 1). Mean BMI tended to increase across birth cohorts (from 25.6 ± 3.6 to 27.0 ± 4.0 kg/m^2) whereas a slight decrease in mean fat intake was observed over time. The percent of subjects with an education above the basic level in the latest birth cohort (40.1%) was more than doubled compared to the earliest one (17.6%). Again, comparing earlier vs. latest born cohorts, subjects who never smoked decreased from 50.3 to 45.8%. Finally, the prevalence of alcohol use was 57.4% in the earliest birth cohort and 87.9% in the latest one. Since no interaction was found between sex and the two dietary exposure variables, the descriptive data in Table 1 is aggregated by sex. Daily intakes of each food group included in the score calculations are depicted in Supplementary Table 1, stratified by both sex and birth cohort.

Table 1 (new): Descriptive analyses of the sample study stratified by birth cohorts. *p* for trends were calculated from unadjusted linear models. SD = Standard Deviation

	1901 (***n*** = **324**)	1911 (***n*** = **232**)	1922 (***n*** = **164**)	1930 (***n*** = **496**)	*p* for trend
Women/men	158/166	115/117	116/48	291/205	< 0.001
Follow-up years to mortality (mean ± SD)	12.9 ± 7.4	13.4 ± 7.1	15.8 ± 6.2	12.7 ± 3.0	0.98
BMI (kg/m^2, mean ± SD)	25.6 ± 3.6	26.5 ± 4.1	26.1 ± 3.8	27.0 ± 4.0	< 0.0001
Total energy intake (kcal, mean ± SD)	2074.5 ± 464.0	2195.3 ± 513.1	2021.9 ± 465.0	2145.7 ± 521.0	0.35
Fat intake (%, mean ± SD)	36.5 ± 5.1	37.5 ± 5.1	35.3 ± 6.0	34.9 ± 6.0	< 0.0001
Protein intake (%, mean ± SD)	3.6 ± 0.5	3.5 ± 0.6	4.1 ± 0.7	4.0 ± 0.6	< 0.0001
Alcohol users (%)	57.4	70.7	80.5	87.9	< 0.0001
Alcohol intake among users (mean ± SD)	5.3 ± 4.7	8.3 ± 11.4	6.4 ± 9.7	9.4 ± 12.1	< 0.0001
Cheese intake (g/day, mean ± SD)	31.4 ± 20.3	33.6 ± 23.8	44.5 ± 34.6	38.9 ± 26.5	< 0.01
Fat from cheese (g/day, mean ± SD)	10.3 ± 5.8	10.6 ± 7.2	11.6 ± 9.0	11.6 ± 9.6	0.02
Milk product intake (g/day, mean ± SD)	369.9 ± 236.9	437.9 ± 240.5	347.0 ± 219.6	341.8 ± 264.6	< 0.001
Fat from milk products (g/day, Mean ± SD)	8.2 ± 6.8	9.4 ± 6.7	5.1 ± 4.2	5.1 ± 5.3	< 0.0001
Low physical activity (%)	14.5	23.3	16.5	7.7	< 0.0001
Education above basic level (%)	17.6	24.1	32.9	40.1	< 0.0001
Never smokers (%)	50.3	45.3	47.6	45.8	0.25
Married (%)	62.7	65.1	46.3	60.1	0.3

Table 2 Association between cheese intake (10 g/day) and milk products (100 g/day) with all-cause mortality, assessed in a Cox regression proportional hazard models both for the total follow-up duration and stratified by duration of follow-up

Follow-up duration	Cases	Cheese		Other dairy products	
		Basic model[1] HRs (95% confidence limits)	Adjusted model[2] HRs (95% confidence limits)	Basic model[1] HRs (95% confidence limits)	Adjusted model[2] HRs (95% confidence limits)
12 years	411	0.93 (0.90; 0.97)***	0.94 (0.91; 0.98)**	1.04 (1.00; 1.08)	1.04 (1.00; 1.08)
20 years	728	0.96 (0.94; 0.99)**	0.97 (0.94; 1.00)*	1.04 (1.00; 1.07)*	1.04 (1.01; 1.08)*
32 years	831	0.96 (0.94; 0.98)**	0.97 (0.94; 0.99)*	1.03 (1.00; 1.07)*	1.04 (1.01; 1.08)**
Total	833	0.91 (0.86; 0.97)**	0.92 (0.87; 0.98)**	1.06 (1.00; 1.13)*	1.06 (1.00; 1.12)‡

The analyses on the total follow-up duration were obtained from models including an interaction term between exposure and follow-up time

$^{*}p$ value < 0.05, $^{**}p$ value < 0.01, $^{***}p$ value < 0.001, $^{‡}p = 0.07$

[1]Adjusted for sex and birth cohort (included as a stratification variable)

[2]Adjusted for sex, birth cohort (included as a stratification variable), smoking status, BMI, education, marital status, physical activity and total energy intake

Cheese and milk product intakes and all-cause mortality

Table 2 shows the association between intakes of cheese and milk products in relation to all-cause mortality, in either crude or fully adjusted models stratified by the duration of follow up. Cheese intake was inversely associated with mortality and the strength of this association tended to decrease with longer follow-up times. On the other hand, milk product intakes showed a positive association with mortality that did not seem to be influenced by follow-up duration. These results were confirmed both by excluding the first 2 years of follow-up and by estimating missing values for education and physical activity by multiple imputation.

Table 3 shows associations across three tertiles of cheese and milk product intakes at different follow up times, adjusted for the above confounders. The association between cheese and mortality was stable across tertiles, whereas a tendency for a dose–response effect was observed for milk products, particularly at the longest follow-up.

When testing non-linearity in piecewise models, we found that cheese intake had an almost constant inverse association with mortality across intake levels, whereas the association of milk products with mortality was mainly driven by the highest intake levels, with the most marked increase in

Table 3 Association between intakes of cheese and milk products with all-cause mortality, across increasing sex-specific tertiles of intakes and at different follow-up durations, assessed in Cox proportional hazard models

Follow-up duration	Cases/tot. subjects	HR (95% confidence intervals)[1]			*p* for trend
		Cheese			
		Low *M* 0–28.7 *F* 0–21.4	Med *M* 3.0–45.0 *F* 21.5–44.5	High *M* > 45.0 *F* > 45.0	
12 years	411/1213	1	0.87 (0.69; 1.09)	0.80 (0.61; 1.03)	n.s
20 years	728/1213		0.92 (0.77; 1.10)	0.92 (0.76; 1.12)	n.s
32 years	831/1213		0.92 (0.78; 1.08)	0.89 (0.74; 1.07)	n.s
Total	833/1213		0.82 (0.65; 1.03)	0.71 (0.48; 1.04)	n.s
Follow-up duration	Cases/tot. subjects	Other dairy products			*p* for trend
		Low *M* 0–286 *F* 0–210	Med *M* 290–475 *F* 214–400	High *M* > 500 *F* > 403	
12 years	411/1213	1	1.03 (0.80; 1.32)	1.08 (0.84; 1.39)	n.s
20 years	728/1213		0.98 (0.82; 1.19)	1.17 (0.97; 1.42)	n.s
32 years	831/1213		1.09 (0.91; 1.29)	1.20* (1.00; 1.44)	< 0.05
Total	833/1213		1.04 (0.81; 1.32)	1.09 (0.75; 1.59)	n.s

Tertile cut-offs are reported in g/day for both males (*M*) and females (*F*). The analyses on the total follow-up duration were obtained from models including an interaction term between exposure and follow-up time

n.s. not significant

*p value < 0.05

[1]Adjusted for sex, birth cohort (included as a stratification variable), smoking status, BMI, education, marital status, physical activity and total energy intake

mortality among those drinking > 800 g/day of milk products. These subjects were mostly sedentary, unmarried men, with higher BMI and high energy intake. Similar results were not found for cheese. Using the likelihood ratio tests, we compared the piecewise model with two models which included either cheese and milk product intakes as continuous variables, or quadratic terms of the intake variables. The likelihood ratio tests were all non-significant.

No interaction between cheese and milk product intakes in relation to their association with mortality was found by likelihood ratio test. The two variables showed a very low correlation with each other ($r = 0.04$) and, when they were included in the same model, their associations with mortality remained unchanged. Associations with mortality of cheese and milk products were not modified by alcohol intake.

A high protein intake was found to modify the association between cheese intake (10 g/day) and all-cause mortality (p for interaction < 0.05) in a way that the latter association was statistically significant at higher (HR = 0.92, 95% CI 0.88; 0.96, $p < 0.0001$) but not at lower protein intakes (HR = 1.00, 95% CI 0.97; 1.05). No evidence for a similar effect modification was found for milk products. Protein intake was positively correlated with BMI ($r = 0.10$, $p < 0.001$).

Dairy fat intake and all-cause mortality

Fat intake from cheese was inversely associated with all-cause mortality (HR = 0.86, 95% CI 0.78; 0.95). This association did not materially change when the model was adjusted for either total fat or total energy intake. Fat intake from milk products showed a non-statistically significant positive association (HR = 1.09, 95% CI 0.97; 1.22), which became significant after adjustment by either total fat or energy intake (HR = 1.18, 95% CI 1.04; 1.34). Neither type of fat was related to BMI (data not shown).

Discussion

The present paper investigated the association of cheese and milk products (i.e. fermented and non-fermented milk) with all-cause mortality and found that cheese intake was negatively associated with mortality, in line with previous results from other two large Swedish studies [11, 12]. In the present study, the association between cheese and mortality did not show any dose–response and maintained statistical significance despite somewhat decreased strength along the follow-up. In addition, this association seemed to be modified by

high protein intake, but not by weight status. A progressive decrease in strength along a lengthy follow-up has already been observed in nutritional epidemiology for other types of associations, for instance in relation to serum vitamin D levels [26, 27] as well as in dietary exposures [28]. Such attenuation in prognostic value of single assessments over time may be attributed to changes in exposure during the follow-up [29].

Since the effects of dairy products on health have traditionally been linked to fat content, we also tested the association between fat from either cheese or milk products and mortality. The result was that the intake of fat from cheese was inversely associated with all-cause mortality, whereas the intake of fat from milk products showed a weak tendency toward a positive association. If dairy fat explained the results obtained here, we would have expected that the intakes of fat from cheese as well as fat from milk products to have similar associations with mortality. Instead, both types showed essentially the same association as the type of dairy they were contained. Therefore, no clear conclusion could be drawn regarding the role of fat from milk products which seemed to be more related to total energy intake. Notably, in our recent study [12], which was based on a larger adult Swedish population, the intakes of low-, medium- and high-fat non-fermented milk were all positively associated with mortality, although high-fat milk intake showed the highest HR.

A potential explanation of the opposite associations of both cheese and cheese fat intakes with mortality compared to milk products might involve the production of healthy bioactive compounds during milk fermentation [30], including some types of healthy saturated fats [31]. In line with this hypothesis, yoghurt consumption has been found to be associated with a healthy weight status and the prevention of type 2 diabetes [32, 33]. Cheese is a source of vitamin K2 (menaquinone) [34] which has been previously associated with a lower risk of cardiovascular disease [35] and which may have contributed to the reduction of mortality risk among cheese consumers. Finally, according to a recently proposed alternative hypothesis, milk fermentation might have health benefits through reducing galactose concentration in milk and the higher oxidation rate related to galactose intake [24].

Although we could not specifically compare fermented vs non-fermented milk, it is interesting to mention that National Statistics show that the per capita availability of non-fermented milk was predominant over fermented milk across the years when our study participants were interviewed (% of fermented milk between $<5\%$ in 1960 to 21% in 2000) [36]. Therefore, we can prudently assume that the results we obtained for combined milk, sour milk and yoghurt could be attributed mostly to milk intake, and that the hazard ratios related to milk intake might have been diluted by a growing availability of fermented milk during the latest recruitment period.

The controversy surrounding health effects of dairy products is not new. An example of this is the evidence for a protective effect of dairy product intake on colon and bowel cancer that contrasts with the positive (although weak) association with prostate cancer [37]. However, there are reasons to believe that the role of dairy intake in elderly Swedes born between 1901 and 1930 may be particularly relevant to investigate. In the period between the 1930s and 1950s, the Swedes had high milk intakes, as a consequence of a previously mentioned campaign approved by doctors, teachers, public health authorities and other experts, and aimed to promote the health effects of milk. In particular, the lobby organization "Mjölkpropagandan" (Milk propaganda), founded in 1923, is probably one of the main reasons why many Swedes (and particularly our study participants, who were young at that time) still view milk as a very healthy food and a good source of nutrients. Milk was often assumed to be an effective way to prevent undernourishment, which had a high prevalence at the time when many of the subjects belonging to this study were born [10].

In our previous paper about the Mediterranean diet in the same population studied here, all dairy products were aggregated and scored as unhealthy foods. In that analysis they were positively associated with mortality, in contrast to the present analyses which highlighted a potentially opposite association between cheese and milk products in relation to all-cause mortality. The present results show the need to disaggregate cheese from milk products when testing the association between the Mediterranean Diet Score, the Healthy Nordic Diet score, and other a priori dietary scores used in epidemiological studies. In particular, since dairy is a typical Nordic food group, the definition of the Nordic Healthy Diet should take into consideration the role of dairy type as well as dairy fat content. Also, it is interesting that the association between milk and mortality, which has been tested in several studies from around the world [13], has only been observed in two big Swedish studies [11, 12]. However, the opposite associations found for cheese and milk products in relation to mortality, may have relevance beyond Sweden.

Our study has both strengths and limitations, the former being the high quality of nutritional data obtained by a diet history during a face to face multi-pass interview with the dietician, validated by the high EI/BMR ratio [17, 19]. Also, although the results cannot be generalized to the whole population, we believe that the initially high response rates (in both men and women) [15] and the homogeneous age at baseline allow us to consider our results generalizable at least to the Swedish population aged 70. The decline in participation rates in this study has been discussed by Eiben et al. [14], who tested whether non-participants differed in any measurable way from participants. Specifically, similar

values for self-rated health, history of myocardial infarction, smoking status and diabetes incidence were found, although unmarried men were significantly under-represented.

The limitations of this study include lack of repeated dietary assessments, small sample size and the fact that milk products could not be analysed separately. It is also worth mentioning that, although the analyses were always adjusted for birth cohort, the results could still be influenced by a residual cohort effect, as the examinations spanned over a large range of time during which living conditions changed in numerous ways. However, when comparing subjects who were born in Sweden in 1901 vs those born 1930, the Swedish National Bureau of Statistics reports that life expectancy at 65 years of age did not differ greatly [38]. Rather, it is the life expectancy at birth which has increased substantially, due to the decrease in infant infections and mortality at early ages, which presumably could not have influenced the results of this study.

We conclude that milk and cheese products complicate the application of a Nordic healthy diet concept in epidemiology. Future studies should address the role of cheese and milk product intake in relation to longevity not only in older adults, but also across the life course.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no competing interests.

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SUPPLEMENTARY INFORMATION

Description of the Idefics consortium: The IDEFICS Consortium includes: the Bremen Institute for Prevention Research and Social Medicine (Germany) (project coordinator), the Ghent University (Belgium), the Research and Education Institute of Child Health (Cyprus), the Copenhagen Business School (Denmark), the National Institute for Health Development (Estonia), the University Joseph Fourier (France), the University of Bremen (Germany), the Technologie-Transfer-Zentrum Bremerhaven (Germany), the University of Glasgow (UK), the Lancaster University (UK), the University of Pécs (Hungary), the University Cattolica del Sacro Cuore (Italy), the National Research Council (Italy), the National Cancer Institute (Italy), the University of Milan (Italy), the University of Zaragoza (Spain), the University of Illes Balears (Spain) and the University of Gothenburg (Sweden).

SUPPLEMENTARY TABLES

Table 1S. Sensitivity analyses aimed to confirm the results from the cross-sectional analysis of the association[a] between high adherence levels to a Mediterranean-like dietary pattern (categorized as fMDS > 3 versus fMDS ≤ 3) and different overweight indicators.

Models	Odds Ratios (95% CIs) of categorical outcomes		β (95% CIs) of continuous outcomes	
	Overweight including obesity[b]	WtHR > 0.5[c]	Baseline waist circumference (cm)	Percent fat mass
Sensitivity analysis 1[d,e]	0.85 (0.76; 0.95)**	1.01 (0.88; 1.14)	-0.12 (-0.32; 0.08)	-0.16 (-0.31; -0.01)*
Sensitivity analysis 2[d,f]	0.85 (0.77; 0.95)**	0.97 (0.86; 1.11)	-0.22 (-0.50; 0.05)	-0.25 (-0.48; -0.02)*
Sensitivity analysis 3[d,g]	0.85 (0.77; 0.94)**	0.99 (0.89; 1.12)	-0.19 (-0.45; 0.07)	-0.20 (-0.41; -0.01)*
Sensitivity analysis 4[d,h]	0.85 (0.77; 0.94)**	1.00 (0.89; 1.13)	-0.17 (-0.43; 0.09)	-0.20 (-0.41; -0.01)*

[a]From logistic and linear regression analyses, of the OR = Odds Ratio; CI = Confidence Interval. [b]Defined according to Cole[27] and compared with the rest of the population, [c]Compared with subjects characterized by WtHR ≤ 0.5. [d]Adjusted for sex, age, study centre, parental education and income. [e]Excluding the highest 5% of BMI, WtHR, waist circumference or percent fat mass (according to the specific analysis). [f]Also adjusted for the proportion of meals consumed at home. [g]Also adjusted for having at least one immigrant parent. [h]Also adjusted for the average number of hours per day the children spent either playing outdoors or in sport clubs. *$p \leq 0.05$, **$p \leq 0.01$.

Table 2S. Sensitivity analyses aimed to confirm the results from the longitudinal analysis of the association[a] between baseline high level of adherence to a Mediterranean-like dietary pattern and the highest quintile of change from baseline to follow up in age, BMI z-score, WtHR, waist circumference and percent fat mass.

Models	Odds Ratios (95% CIs)			
	Highest quintile of BMI z-score change	**Highest quintile of WtHR change**	**Highest quintile of waist circumference change**	**Highest quintile of percent fat mass change[b]**
Sensitivity analysis1[c,d]	0.88 (0.78; 0.98)*	0.87 (0.77; 0.98)*	0.87 (0.77; 0.98)*	0.89 (0.78; 1.01)
Sensitivity analysis 2[c,e]	0.86 (0.76; 0.98)*	0.87 (0.77; 0.99)*	0.88 (0.77; 1.00)*	0.91 (0.79; 1.04)
Sensitivity analysis 3[c,f]	0.87 (0.78; 0.98)*	0.88 (0.78; 0.98)*	0.87 (0.77; 0.98)*	0.89 (0.79; 1.01)
Sensitivity analysis 4[c,g]	0.86 (0.77; 0.97)*	0.87 (0.78; 0.98)*	0.87 (0.77; 0.98)*	0.87 (0.76; 0.98)*

[a]From logistic regression analyses. [b]Mean difference at follow up: 1.8% ± 3.7. [c]Adjusted for baseline BMI z-scores, WtHR, waist circumference, fat mass (according to the analyses), as well as sex, age, study center, parental education and income. [d]Excluding the highest 5% of BMI, WtHR, waist circumference or percent fat mass (according to the specific analysis). [e]Also adjusted for the proportion of meals consumed at home. [f]Also adjusted for having at least one immigrant parent. [g]Also adjusted for the average number of hours per day the children spent either playing outdoors or in sport clubs. *$p \leq 0.05$.

1. **How often does your child usually eat at home or at other people's home (e.g. grandparents, friends)?** Please tick for every meal.

	Daily	*Only at weekdays*	*Only at weekends*	*Several times per week*	*On fewer occasions*
Breakfast	❍1	❍2	❍3	❍4	❍5
Lunch	❍1	❍2	❍3	❍4	❍5
Dinner	❍1	❍2	❍3	❍4	❍5
Other country-specific food occasions	❍1	❍2	❍3	❍4	❍5
Other, please specify:_____	❍1	❍2	❍3	❍4	❍5

2. **How would you describe mealtimes with your child?**

❍1 Always pleasant
❍2 Usually pleasant
❍3 Sometimes pleasant
❍4 Never pleasant

3. **Below you find some general statements regarding the diet of children.** Please mark the statements to which you agree.

❍1	A good way to get a child to finish a chore is to promise a snack when he/she is finished.
❍1	Children should have dessert only after everything on the plate has been eaten.
❍1	It is all right to tell a child he/she can't have dessert because he/she misbehaved.
❍1	When children feel sad or "blue", a favourite food will make them feel better.
❍1	Just because a school age child is under pressure, it is not an excuse for eating extra sweets.
❍1	A parent who loves his child never keeps food from him/her.
❍1	Food has little to do with a good parent-child relationship.
❍1	A good way for a parent to show love for his/her child is to buy him/her a "sweet treat".

4. **How often does your child eat doing something else (e.g. watching TV, playing, sitting at a computer, looking at a book)?**

❍1 Never or rarely
❍2 Several times per week
❍3 Once a day
❍4 On several occasions per day

5. **Does your child habitually receive a particular feeding pattern?**

	Yes	*No*
Without meat, poultry and sausage	❍1	❍2
Without fish	❍1	❍2
Without milk and milk products	❍1	❍2
Without eggs	❍1	❍2
Other, please specify: _____	❍1	❍2

6. **How many times does your child eat fast food in a fast food restaurant (<*please insert country-specific examples*> etc.) or at stands or kiosks (<*please insert country-specific examples*> etc.)...**

	Never	*Once a month or less*	*Several times a month*	*1-2 times a week*	*3 or more times a week*
... to consume a full meal alternative to a normal meal (breakfast, lunch, dinner)?	❍1	❍2	❍3	❍4	❍5
... to consume some food as snack between meals?	❍1	❍2	❍3	❍4	❍5

7. Please mark for each of the following statements how much it applies to your child.

	Never	*Rarely*	*Sometimes*	*Often*	*Always*
Does your child watch TV at meals?	❍1	❍2	❍3	❍4	❍5
Is it a struggle to get your child to eat?	❍1	❍2	❍3	❍4	❍5
Do you feed your child yourself if he/she does not eat enough?	❍1	❍2	❍3	❍4	❍5
Do you have to stop your child from eating too much?	❍1	❍2	❍3	❍4	❍5
Do you think about putting your child on a diet to keep him/her from becoming overweight?	❍1	❍2	❍3	❍4	❍5
Do you make your child eat all the food on his/her plate?	❍1	❍2	❍3	❍4	❍5
Do you worry that your child is eating too much?	❍1	❍2	❍3	❍4	❍5
Do you worry that your child is eating not enough?	❍1	❍2	❍3	❍4	❍5
Do you use foods that your child likes as a way to get your child to eat "healthy" foods he/she does not like?	❍1	❍2	❍3	❍4	❍5
Does your child have a poor appetite?	❍1	❍2	❍3	❍4	❍5
Do you sit down with your child when he/she eats meals?	❍1	❍2	❍3	❍4	❍5

8. Are sweetened soft drinks available at home during meals?

❍1 Yes, often or always
❍2 No or seldom

9. Please mark how much you agree or disagree with the following statements.

	Disagree	*Moderately disagree*	*Unsure*	*Moderately agree*	*Agree*
I compare labels to select the most nutritious food.	❍1	❍2	❍3	❍4	❍5
I have more confidence in food products that I have seen advertised than in unadvertised products.	❍1	❍2	❍3	❍4	❍5
I try to avoid food products with additives.	❍1	❍2	❍3	❍4	❍5
I make a point of using natural or ecological food products.	❍1	❍2	❍3	❍4	❍5
I prefer to buy meat and vegetables fresh rather than pre-packed.	❍1	❍2	❍3	❍4	❍5
We use a lot of ready-to-eat foods in our household.	❍1	❍2	❍3	❍4	❍5
I use a lot of mixes, for instance baking mixes and powder soups.	❍1	❍2	❍3	❍4	❍5
The kids help in the kitchen, e.g. they peel the potatoes and cut the vegetables.	❍1	❍2	❍3	❍4	❍5

10. In the last month, how many times did your child eat or drink the following food items? Please refer to the last four weeks and exclude foods served at school.

	Never / < once a week	**1 - 3 times a week**	**4 - 6 times a week**	**1 time per day**	**2 times per day**	**3 times per day**	**4 or more times per day**	**I have no idea**
Vegetables								
Cooked vegetables, potatoes and beans (also in mixed recipes)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Legumes (only in Greece)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Fried potatoes, potato croquettes	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Raw vegetables (mixed salad, carrot, fennel, cucumber, lettuce, tomato)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8

	Never / < once a week	1 - 3 times a week	4 - 6 times a week	1 time per day	2 times per day	3 times per day	4 or more times per day	I have no idea
Fruits								
Fresh fruits (also freshly squeezed, fruit smoothie) ***without*** added sugar	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Fresh fruits (also as freshly squeezed, fruit smoothie) ***with*** added sugar	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Drinks								
Water	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Fruit juices (orange juice, apple juice, *local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Sweetened drinks including sports drinks, bottled or canned tea, syrup-based drinks and similar (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Diet coke or diet soft drinks (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Breakfast cereals								
Sweetened or sugar added breakfast cereals and sweetened crisp muesli (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Porridge, oat meal, gruel, unsweetened cereals, plain muesli (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Milk								
Plain unsweetened milk	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Sweetened milk (e.g. addition of sugar, chocolate powder, honey etc.)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
What kind of milk does your child usually consume:	❍1 whole ❍2 semi-skimmed / skimmed							
Yoghurt								
Plain unsweetened yogurt or kefir (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Sweet yoghurt and fermented milk beverages (e.g. Actimel®, LC1®, *local examples,* etc.)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
What kind of yoghurt does your child usually consume:	❍1 whole ❍2 semi-skimmed / skimmed							
Fish								
Fresh or frozen fish, not fried	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Fried fish and fish fingers	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Meat and meat products								
Cold cuts and preserved, ready to cook meat product (please exclude sandwiches (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Fresh meat, not fried (chops, steak, bovine, pork, poultry etc.)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Fried meat (chops, steak, bovine, pork, poultry etc.)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8

Eggs and mayonnaise								
Fried or scrambled eggs	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Boiled or poached eggs	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Mayonnaise and m. based products (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Meat replacement products and soy products								
Tofu, tempé, quorn, soy milk (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Cheese								
Sliced cheese (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Spreadable cheese (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Grated cheese (only in Italy)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Spreadable products								
Jam, honey	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Chocolate or nut-based spread	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Butter, margarine on bread	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Reduced-fat products on bread (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Ketchup (also as a topping on fries)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Cereal products								
White bread, white roll, white crispbread	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Whole meal bread, dark roll, dark crispbread	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Pasta, noodles, rice	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Dish of milled cereal (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Pizza as main dish	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Hamburger, hot dog, kebab, wrap, falafel, *local examples*	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Snacks								
Nuts and seeds and dried fruits (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Snacks like crisps, corn crisps, popcorn etc (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Snacks like savoury pastries and fritters (e.g. cheese pie, sausage pie, pancakes, pizza as snack, *local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Snacks like chocolate, candy bars (mars, lions, kit Kat, *local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Snacks like candies, loose candies, marshmallow (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Snacks like biscuits, packaged cakes, or pastries and puddings (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8
Ice cream, milk or fruit based bars (*local examples*)	❍1	❍2	❍3	❍4	❍5	❍6	❍7	❍8

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ORIGINAL ARTICLE

Adherence to a Mediterranean-like dietary pattern in children from eight European countries. The IDEFICS study

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BACKGROUND: Despite documented benefits of a Mediterranean-like dietary pattern, there is a lack of knowledge about how children from different European countries compare with each other in relation to the adherence to this pattern. In response to this need, we calculated the Mediterranean diet score (MDS) in 2–9-year-old children from the Identification and prevention of dietary- and lifestyle-induced health effects in children and infants (IDEFICS) eight-country study.

SUBJECTS AND METHODS: Using 24 h dietary recall data obtained during the IDEFICS study ($n = 7940$), an MDS score was calculated based on the age- and sex-specific population median intakes of six food groups (vegetables and legumes, fruit and nuts, cereal grains and potatoes, meat products and dairy products) and the ratio of unsaturated to saturated fats. For fish and seafood, which was consumed by 10% of the population, one point was given to consumers. The percentages of children with high MDS levels (>3) were calculated and stratified by sex, age and by having at least one migrant parent or both native parents. Demographic (sex and age) and socioeconomic characteristics (parental education and income) of children showing high (>3) vs low ($\leqslant 3$) MDS levels were examined.

RESULTS: The highest prevalence of children with MDS >3 was found among the Italian pre-school boys (55.9%) and the lowest among the Spanish school-aged girls (26.0%). Higher adherence to a Mediterranean-like dietary pattern was not associated with living in a Mediterranean country or in a highly educated or high-income family, although with some exceptions. Differences in adherence between boys and girls or age groups varied between countries without any general pattern.

CONCLUSIONS: With the exception of Italian pre-schoolers, similar adherence levels to a Mediterranean-like dietary pattern have been observed among European children.

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INTRODUCTION

The traditional Mediterranean diet is characterised by a high intake of vegetables, legumes, fruits, nuts and cereal grains (largely unrefined in the past), moderate-to-high fish intakes, high intakes of unsaturated lipids (particularly from olive oil) but low intakes of saturated fats, a low-to-moderate intake of dairy products (mostly cheese and yoghurt) and finally, a low intake of meat products.[1,2]

In adults, a Mediterranean-like dietary pattern is known to be inversely associated with mortality,[3] as well as with a number of chronic diseases including cardiovascular diseases,[4] cancer,[5,6] obesity,[7] metabolic syndrome[8,9] and also diabetes type 2,[10] probably due to the relatively low glycaemic load in spite of the potentially high carbohydrate content.[11] This dietary pattern has also been suggested to improve cognition[12] and to increase longevity[13] and appears to be associated with a better health status overall.[14] Such benefits have been observed both in Mediterranean[15] as well as in non-Mediterranean countries.[16]

Adherence to a Mediterranean-like dietary pattern has often been evaluated based on scores. The most commonly applied Mediterranean diet score (MDS) used in studies on adults was developed by Trichopoulou *et al.*[17] The score was subsequently modified to include fish[18,19] or calculated based on tertile rather than median intakes for application in large population studies.[20] The MDS is often calculated by allocating one point each when the energy-adjusted intakes for any of the most common Mediterranean foods (vegetables, legumes, fruit, nuts and so on) or the ratio of unsaturated to saturated fats is above the population median, as well as by allocating one point each when the intakes for any food that is atypical of the Mediterranean pattern (meat and dairy products) is below the population median.

In children and adolescents, scores have been developed to measure adherence to a Mediterranean-like diet, but they have usually been applied in children from Mediterranean countries, with few exceptions, that is, Mexico,[21] UK,[22] and a multi-centre study including 20 countries.[23] Another household-based study[24] was performed in Portugal, which is considered very close to the Mediterranean countries concerning dietary habits.[25] A number of scoring systems have been used to assess the adherence to a Mediterranean-like dietary pattern in children and adolescents. These include the above-mentioned MDS[3,26] as well as a similar

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score developed by Garcia-Marcos *et al*.[27] The latter, in particular, was based on a three-level scoring system where food intake frequency was rated 0, 1 or 2 from less to more typical Mediterranean foods and from more to less atypical foods. Finally, Serra-Majem *et al*.[28] developed the Mediterranean Diet Quality Index for children and adolescents (generally referred to as KIDMED), based on 16 different frequency questions of food intake.

We have recently published results based on a 43-item food frequency questionnaire (FFQ) of the eight-country cohort study Identification and prevention of dietary- and lifestyle-induced health effects in children and infants (IDEFICS), where we applied a version of the MDS adapted to food frequencies (rather than quantities) adjusted for the total daily consumption frequency of all food items in the FFQ.[29] Our results showed an unexpected distribution of the adherence levels to a Mediterranean-like pattern: the Swedish children had the highest scores and the Cypriot children had the lowest scores. As our previous score was based on usual frequencies of consumption of a limited number of food items in each food group, in this paper we present descriptive analyses of adherence levels to a Mediterranean-like dietary pattern based on food quantities assessed using a computer-based 24 h dietary recall and including a higher number of food items.

SUBJECTS AND METHODS

Study subjects

A population-based cohort of 16 228 children (response proportion 53.4%) aged 2–9 years was examined in a baseline survey in eight European countries ranging from North to South and from East to West (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium and Estonia) from autumn 2007 to spring 2008. The sample of the present analysis was taken from the baseline survey (T_0) of the IDEFICS study, which includes the largest European children's cohort established to date.[30]

All 2–9-year-old children who resided in the defined regions and attended the selected primary schools (grades 1 and 2) or pre-schools were eligible for participation and were approached via schools or pre-schools. In addition to the signed informed consent given by parents, each child was asked to give verbal assent immediately before examination. Participants were free to opt out of specific modules (for instance, blood collection).[30]

Questionnaires

The parents completed a self-administered questionnaire to assess behavioural and sociodemographic factors. Parental education, employment status, dependence on social welfare and migration background of parents were recorded. Educational attainment was defined according to the International Standard Classification of Education,[31] whereas family income was categorised using country-specific categories based on the average net equivalence income.

The survey also included a Children's eating habits questionnaire to describe food frequencies and dietary habits over the last month, completed by the parents of 14 972 children (49.1% girls). This was complemented by a computer-based 24 h dietary recall, namely, Self-Administered Children and Infant Nutrition Assessment (SACINA), which produced the data used in the present study. SACINA was recently validated with doubly-labelled water, showing to be a valid instrument to assess energy intake at group level.[32] The SACINA was based on the previous designed and validated YANA-C (Young Adolescents' Nutrition Assessment on Computer) developed for Flemish adolescents and further adapted to European adolescents in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study.[33] The SACINA software was developed to assess children's absolute nutrient and energy intake, the contribution from food and drinks to total energy and nutrient intake, as well as portion sizes and food groups, during the previous 24 h. It was structured according to six meal occasions: breakfast, mid-morning snack, lunch, afternoon snack, evening meal and evening snack. Choice of portion size was assisted by the display of photographs of selected food items, many of which were country-specific. The parents or other proxies completed the 24 h dietary recall under the supervision of fieldwork personnel in about 20–30 min. Except for Cyprus, where school ends before lunch, school and pre-school meals were additionally assessed by means of direct observation. Teachers and school kitchen staff were interviewed by trained survey personnel and data were collected using special documentation sheets, including portion sizes. School and pre-school meal data were merged with parentally reported data to enhance completeness of dietary intakes. The assessment procedure in Hungary differed slightly from the other study centres. Here all dietary information was recorded on documentation sheets and entered into the SACINA program afterwards. The coded food items were linked to country-specific food composition tables.

A total of 14 863 recall interviews have been done. Of these, 11 669 were first recalls (about 78% of the whole IDEFICS cohort). Exclusion criteria included incomplete interviews ($n = 1913$; for example, when the proxy did not know about at least one main meal or in case of no school or pre-school meal information) as well as recalls characterised by implausible intakes (for example, $>85\%$ energy from fat) or by under-reporting or over-reporting ($n = 1816$), the latter two identified according to Goldberg cut-offs adapted to children as reported in a previous publication.[34] The final data set after exclusions included 7940 first recalls (6738 on work days and 1202 on weekends) as well as 2219 second recalls (1443 on work days). However, due to missing values in the variables related to the parental socioeconomic and migration status, the above numbers varied in some analyses. Data from second recalls were not used in the main analysis, but only in a supplementary analysis, due to the limited number. Also, the procedures for the estimation of usual intakes were not stable when being applied to single (non-daily) consumed food items, that is, the models did not converge. We also decided not to use the mean of both recalls (where available) to avoid differing numbers of recalls between the children.

Food groups and MDS

On the basis of the SACINA data, daily intakes (g per day) of all food items were calculated and six food groups were created: (1) vegetables and legumes; (2) fruit and nuts; (3) cereal grains and potatoes; (4) fish products; (5) meat products; (6) dairy products (see Appendix for details about the foods included in each food group); and (7) a ratio of unsaturated (that is, the sum of mono- and polyunsaturated fats: monounsaturated fatty acids +polyunsaturated fatty acids) to saturated fats. The decision to include both types of unsaturated fats was based on the fact that polyunsaturated fatty acids, and not only monounsaturated fatty acids, are the principal unsaturated fats in non-Mediterranean diets.[35] Food intakes from soups were divided by two to consider the contribution of water to the food weight. Intakes were standardised to an intake level of 1000 kcal and medians were calculated by the children's sex and age categorised as pre-school (<6-year olds) and school age ($\geqslant 6$-year olds). One point was given for individual intakes lying above the food group median for vegetables and legumes, fruit and nuts as well as for cereal grains and potatoes. For the unsaturated to saturated fat ratio, one point was given when children were above the median. For meat and dairy products, one point was given when the child's intake was below the median. Finally for fish, which was consumed by only 8.7% of the children on the single recall day, one point was assigned to consumers. The sum of these points gave a seven-point MDS, and high adherence to a Mediterranean-like dietary pattern was considered when the score was >3 as in previous analyses based on the FFQ data.[29]

Statistical analyses

Prevalence of high adherence to a Mediterranean-like dietary pattern (defined as MDS >3) was calculated both for the whole sample and stratified by participating centre, sex, age group and by parental migration status, as previously described. Due to the limited number of recalls collected during weekend days (that is, Saturday and Sunday, 15.8% of all recalls), the prevalence of high adherence during these days was not reported, stratified by sex, age or parental migration status. In addition, the proportion of boys, pre-school age children, as well as the prevalence of highly educated and higher-income parents and of children having at least one migrant parent were calculated both among children with high (MDS >3) and low (MDS $\leqslant 3$) adherence. The prevalence of high adherence from the second dietary recall was also calculated for the overall sample, as well as stratified by sex and age and based on work days. In addition, a version of the MDS excluding foods consumed at school or pre-school was calculated. A second alternative version of the MDS was obtained by

excluding potatoes from the score calculations and the prevalence of high adherence was calculated, stratified by sex and age group.

All statistical analyses were carried out using the SAS statistical software version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Table 1 describes the prevalence of high adherence to a Mediterranean-like dietary pattern (that is, MDS > 3) stratified by participating centre, sex, age group (age < or ⩾6 years) and by having at least one migrant parent or both native parents. During work days, in both age groups and sexes, the Italians (together with Spanish school-age boys) showed the highest prevalence of high-adherent children, whereas the lowest adherence levels (<30%) were observed in Estonian (pre-school age), German and Cypriot (school age) boys, as well as in German (pre-school age), Spanish (school age) and Cypriot girls. With the exception of Estonia, children having at least one migrant parent had higher levels of adherence. Figure 1 summarises the prevalences of high adherence (MDS > 3) by age group and country, showing that the highest adherence levels were found in Italian pre-school children.

Different food groups contributed to high adherence levels in each country as can be shown by the prevalence of children whose intakes were above (high consumers) or below (low consumers) the age- and sex-specific median intake for each food group. For instance, although among Italian children we found a low prevalence of high consumers of vegetables and legumes (36.2%), Sweden was characterised by a very low prevalence of high consumers of both dairy (24.6%) and meat (30.9%) products. An increased prevalence of children above the median for the unsaturated:saturated fat ratio in Italy (72.6%) and Cyprus (58.7%) was observed, whereas a high prevalence of high consumers of cereal grains and potatoes characterised both the Swedish (81.7%) and the Estonian (63.1%) sample. Finally, the highest prevalence of fish consumers was detected in Spain (25.8% of consumers).

In general, the adherence during weekend days was higher compared with work days, with the exception of Italy and Spain where the prevalence of high adherence during weekends was about 10% (Spain) or 20% (Italy) lower than that during work days. In Estonia and Hungary, the number of recalls during weekends was too limited to make a comparison with work days.

The comparison with a second recall was possible only for a limited number of centres, since after the application of the exclusion criteria, <30 recalls were available for Estonia, Belgium and Cyprus. For the remaining countries, the results were mainly consistent with the results based on the first recall, with Italy scoring the highest (55% of high-adherent children overall), followed by the Swedish (47% of high-adherent children) and by the Hungarian, German and Spanish children (<35% of high-adherent children; results not shown in the tables).

With the aim of understanding better how school and pre-school meals could influence the adherence to a Mediterranean-like dietary pattern, the MDS was recalculated excluding food items provided at school. In Cyprus, Belgium, Germany and among school-age Italian children, only <5% of food items were consumed at school, so no specific comments about the influence of school and pre-school meals can be made. In summary, in Swedish pre-school children, the adherence levels increased after excluding meals provided by the pre-school, whereas the opposite tendency was observed in school-age children when excluding school and pre-school meals. A decreased adherence after excluding school/pre-school meals was observed in Hungary, Spain and among Italian pre-school children (results not shown in the tables).

A further classification of high-adherent (MDS > 3) and low-adherent (MDS ⩽ 3) children based on sex, age and socioeconomic status showed that both high- and low-adherent children had homogeneous characteristics, with very few exceptions (Table 2).

Finally, since in this analysis potatoes were counted with grains, as opposed to considering them as vegetables, we ran a sensitivity

Table 1. Percentages of high adherence to a Mediterranean-like diet by day of the week, sex, age and parental migrant status

	Northern Europe		*Central Europe*			*Mediterranean Europe*		
	Sweden	*Estonia*	*Hungary*	*Belgium*	*Germany*	*Italy*	*Spain*	*Cyprus*
				Work days (Monday–Friday)				
Number of recalls	910	640	961	287	1 265	1 385	411	879
Boys								
Pre-school	37.4	29.8	31.5	34.3	27.8	55.9	32.3	33.9
School	36.8	30.0	37.7	35.1	29.6	42.5	42.6	28.0
All boys	37.1	29.9	35.3	34.6	28.8	48.6	36.7	30.4
Girls								
Pre-school	36.1	36.0	30.8	35.1	29.1	55.0	37.1	29.5
School	39.8	35.6	35.4	38.6	30.7	46.7	26.0	28.4
All girls	38.1	35.7	33.4	36.6	30.0	50.1	31.6	28.8
Migrant status[a]								
Native parents	35.7	33.1	34.2	34.6	28.7	48.2	33.7	28.3
At least one migrant parent	46.7	23.3	36.2	43.8	31.2	54.5	36.4	30.0
All children	37.6	33.0	34.3	35.5	29.4	49.3	34.3	29.6
				Weekend days (Saturday and Sunday)				
Number of recalls	235	10	32	50	263	367	144	101
All children	47.2	40.0	28.1	30.0	39.5	27	24.3	31.7

Number of recalls during work days and weekend days, as well as percentages of children characterised by a Mediterranean diet score >3 during work days among pre-school (<6-year olds) or school age (⩾6-year olds) children, stratified by country. Because of the smaller number of recalls, no stratification by sex and age was done for weekend days. [a]The parental migration status was missing for some children. The number of missing values was ⩽15 in most of the countries, except for Germany ($n = 32$) and Cyprus ($n = 112$) where a high number of missing values was present.

analysis by excluding them. The results we obtained confirmed what previously observed when including potatoes in the calculations. For instance, in pre-school Italian boys and girls the prevalence of high adherence was >50% and the Spanish school-aged girls scored the lowest as in the main analysis.

DISCUSSION

In this paper, we described the adherence to a Mediterranean-like dietary pattern in 2–9-year-old boys and girls from eight European countries. As previously reported in a paper based on FFQ data,[29] this study confirmed that a Mediterranean-like dietary pattern is not necessarily a feature of the Mediterranean countries and that children from Southern Europe countries can even have lower adherence proportions than their peers living in other geographical areas. This finding raises the question whether we should continue calling this pattern 'Mediterranean-like', also considering that the adherence is evaluated based on a score calculated considering intakes at the group level (vegetables, meat products and so on) and the actual food items feeding into each group could vary from country to country (for example, rye and salmon are more common in Nordic countries than in Southern Europe). As a confirmation of this, what in North Europe has been defined as a Nordic healthy diet (or 'New Nordic diet') was actually inspired by those principles the Mediterranean pattern has been based on.[36]

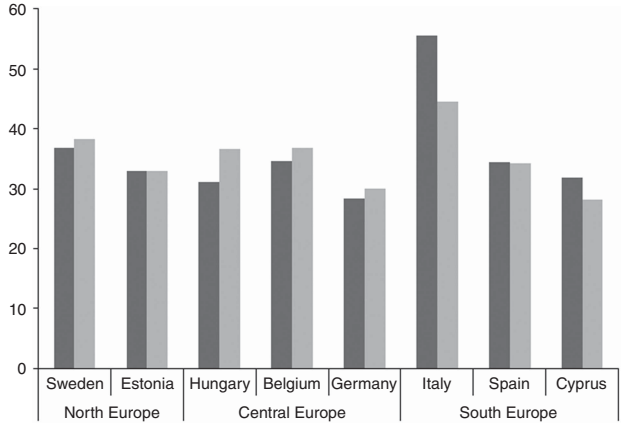

Figure 1. Prevalence of high adherence to a Mediterranean-like dietary pattern (MDS > 3) among pre-school (dark grey) and school children (light grey), stratified by country.

Most of our analyses were based on weekday data but we were also able to make a comparison with weekend days, which showed that the Italians had the highest MDS levels during work days, whereas the Swedish scored the highest during weekend days. However, the possibility of making further speculations on this result was limited by the very small number of recalls done during weekends in some of the countries.

In this study, with the exception of Italy, the proportion of highly educated parents was increased (generally slightly) among high-adherent children, with the biggest difference observed for the Spanish children. The proportion of higher-income parents was increased among high-adherent children in some countries and lower in others. In all the centres, except Estonian children, having at least one migrant parent showed higher adherence levels than those who had both native parents. However, the latter observation can have a different interpretation according to the specific country of residence, as in North Europe a migrant parent might come from a Mediterranean country for instance.

Many variations of the MDS have been adopted in epidemiological studies,[37] therefore the harmonisation of dietary data is mandatory for better comparison of health outcomes.[38–40] Accordingly, we have previously published a paper based on a version of the MDS adapted to the food frequency data used in the IDEFICS study to assess children's usual diets[29] in relation to their weight status. In the present paper, we used data collected in the same study using another methodology, 24 h recall data, which could provide more detailed food and nutrient data but which is less useful than the FFQ for considering associations with health-related outcomes such as body weight. The results obtained from the analysis of the present data highlighted some differences in the ranking of the country-level adherence to a Mediterranean-like dietary pattern. More specifically, Italy has replaced Sweden as the participating centre characterised by the highest adherence levels, but only during work days, whereas the children from Cyprus did not show the lowest adherence levels. However, it has to be pointed out that the present analysis was based on a single day, whereas the FFQ was designed to collect data referring to a broader timespan and only food intakes under parental control, thus excluding school and pre-school meals, which were instead included in SACINA. From a methodological point of view, SACINA is characterised by two major differences compared with the FFQ, that is, the inclusion of meals consumed at school or pre-school and the possibility to calculate the MDS based on food quantities instead of frequencies as well as to include a ratio between unsaturated and saturated fats. Food items consumed at school (or pre-school) contributed to either

Table 2. Distribution of gender, age and socioeconomic status among high- and low-adherent children.

	n	*Northern Europe*		*Central Europe*			*Mediterranean Europe*		
		Sweden	*Estonia*	*Hungary*	*Belgium*	*Germany*	*Italy*	*Spain*	*Cyprus*
Number of low-adherent children (MDS ⩽ 3) (%)		568	429	631	185	893	702	270	619
Boys	4 297	52.2	49.7	49.1	55.4	51.0	52.5	50.6	49.6
Pre-school children	4 297	45.9	35.8	43.1	61.3	43.1	37.7	53.2	37.5
Children with high-education parents	4 154	68.8	12.6	47.9	47.6	19.1	19.7	52.4	53.6
Children with high-income parents	3 821	27.6	40.5	27.7	18.6	5.0	1.3	28.5	9.2
Number of high-adherent children (MDS > 3) (%)		342	211	330	102	372	683	141	260
Boys	2 441	51.5	43.2	50.5	52.5	50.3	52.1	57.8	51.6
Pre-school children	2 441	44.4	35.2	36.5	58.4	40.3	48.8	54.9	41.5
Children with high-education parents	2 365	70.8	14.6	49.2	50.5	21.9	18.9	61.4	54.3
Children with high-income parents	2 129	23.0	45.5	26.3	18.3	6.7	1.7	24.6	9.6

Abbreviation: MDS, Mediterranean diet score. Number of high- and low-adherent children, as well as percentages of boys, pre-school children (<6-year old), high-education and high-income parents among the children characterised by high (>3) or low (⩽3) levels of the MDS during work days.

higher (Italian pre-school children, Spanish and Hungarian children) or lower adherence levels (Swedish school children).

Several recent cross-sectional studies have assessed the adherence to a Mediterranean-like dietary pattern in children or young adolescents by diet scores. Although many of these used the KIDMED score and were based on food frequencies,[28] we decided to use a modified version of the MDS[39,40] readapted to the IDEFICS 24 h dietary recall.[29] The reasons of this choice were twofold: first, this score will give us the possibility to compare the present results with those from future follow-ups when the IDEFICS children will have become adults and the KIDMED will not be applicable. Second, the KIDMED index includes in its scoring criteria some dietary characteristics, which are not specifically associated to the Mediterranean habits, such as having cereals and dairy products for breakfast or having breakfast.[28] Many of the above-mentioned studies on the Mediterranean diet in children described the cross-sectional association with health outcomes such as asthma[41] or overweight.[42] Most were set up in a Mediterranean country such as Greece,[42–44] Cyprus,[45,46] Spain[47,48] and Turkey.[49] A few studies were based on children living in a non-Mediterranean country like UK[22] and Mexico.[26] Moreover, to our knowledge, only the International Study on Allergies and Asthma in Childhood (ISAAC) assessed the adherence of children from different countries, analysing dietary data of children from 29 centres in 20 countries.[23] However, it is difficult to compare our results with this study as they did not calculate the MDS in all countries due to missing data in some of the questionnaires (for instance, the German and Swedish ones) and also because some countries included in our study (that is, Cyprus, Hungary or Belgium) were not part of the ISAAC study. Moreover, the MDS is based on population-specific medians (or consumers/non-consumers as in the case of fish) and not based on specific thresholds, which limits the comparability with other analyses. Therefore, although conclusions based on a single-day analysis should be drawn carefully, we think that it is important to notice that our results confirm what we had previously shown based on FFQ data, that is, that countries outside the Mediterranean basin (for example, Sweden) can have higher adherence levels to a Mediterranean-like dietary pattern than in Southern European countries (for example, Cyprus). High intakes of each food group included in the calculation of the MDS were not a characteristic of any specific geographic area. Therefore, the way each food group contributed to high adherence levels also differed across participating centres.

Strengths of the study include its high number of participants, the fact that we collected data from eight different countries and the possibility to study the distribution of high adherence according to sex, age and socioeconomic features (that is, parental education and income, presence of at least one migrant parent in the family). However, this study is not without limitations. The latter includes the fact that the analyses were based mostly on work days, due to the low number of recalls at weekends and based only on a single-day recall. A single recall may not necessarily reflect habitual intakes such that misclassifications may have occurred especially in case of non-daily-consumed food groups like fish. In Hungary, a further source of error was introduced as the dietary information was recorded on documentation sheets before entering data into the SACINA software, therefore limiting the comparability with the other countries. Finally, due to the clustered study design, the survey cannot be considered representative of the national population and thus we do not know whether the habits of the participants in this study reflect those of the majority of European children. However, the IDEFICS sampling was population-based and the study was not designed to generate a representative sample for the given countries. Rather, it was important to select one intervention and control region that were comparable with regard to infrastructural, sociodemographic and socioeconomic characteristics.[30] The participation proportion of 53.4% may appear to be low and we have no systematic information on non-participants, but, thanks to the community-oriented and setting-based study design, the IDEFICS study approached the whole population for participation.

In conclusion, adherence proportions among European children were found to be homogeneous, with the highest adherence proportions among the Italian pre-school children. The exclusion of potatoes from the group of starchy foods, as they are more typical of central-northern European traditions, did not practically change the main results. Both the demographic and socioeconomic characteristics of high-adherent children slightly differed between countries.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

GT performed the analyses and wrote the paper. LAM, TM, TV, DM, PR, AS, YA, VK, MT, CB, AH provided comments on the manuscript and on the analyses. IP supported the statistical analyses and their interpretation and provided comments on the manuscript. LL coordinated the research, supported the analyses and gave comments on the manuscript.

DISCLAIMER

The information in this document reflects the author's view and is provided as it is.

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APPENDIX

List of foods included in each MDS component.

Foods available in the SACINA database that are typical of a Mediterranean-like dietary pattern

1. Vegetables and legumes
- Tomato and other vegetable-based sauces
- Pulses (excluding fresh peas, sweet corn and broad bean)
- Vegetables/vegetable salad excluding potatoes
- Root vegetables
- Vegetable juices
- Vegetarian burgers, tempeh and tofu
- Vegetable milk (soymilk, rice milk and so on)
- Vegetable soups

2. Fruit and nuts
- Nuts, seeds and olives
- Fruit
- Fruit and vegetable juices—fresh made—squeezed

3. Cereal grains and potatoes
- Bread and rolls
- Breakfast cereals
- Flour—instant-creamed cereals
- Pasta
- Rice and other cereals
- Cereal soups
- Potatoes (excluding fried potatoes and chips)

4. Fish products
- Foods based on fish products
- Fish, crustaceans and molluscs
- Fish products

Foods available in the SACINA database that are atypical of a Mediterranean-like dietary pattern

1. Meat products
- Sauces (savoury)—meat, ragout and gravy
- Meat
- Game
- Offals
- Processed meat—salami and processed poultry products
- Meat soups

2. Dairy products
- Butter–cream–cheese-based sauces
- Milk and similar
- Cheese (excluding 'fromage blanc' (quark))
- Other milk products—NS

9. Appendix 2 – List of ICD codes used in this thesis

ICD-8

390 Rheumatic fever without mention of heart involvement
391 Rheumatic fever with heart involvement
392 Chorea
(393-398) Chronic rheumatic heart disease
393 Diseases of pericardium
394 Diseases of mitral valve
395 Diseases of aortic valve
396 Diseases of mitral and aortic valves
397 Diseases of other endocardial structures
398 Other heart disease, specified as rheumatic
(400-404) Hypertensive disease
400 Malignant hypertension
401 Essential benign hypertension
402 Hypertensive heart disease
403 Hypertensive renal disease
404 Hypertensive heart and renal disease
(410-414) Ischaemic heart disease
410 Acute myocardial infarction
411 Other acute and subacute forms of ischaemic heart disease
412 Chronic ischaemic heart disease
413 Angina pectoris
414 Asymptomatic ischaemic heart disease
(420-429) Other forms of heart disease
420 Acute pericarditis, non-rheumatic
421 Acute and subacute endocarditis
422 Acute myocarditis
423 Chronic disease of pericardium, non-rheumatic
424 Chronic disease of endocardium
425 Cardiomyopathy
426 Pulmonary heart disease
427 Symptomatic heart disease
428 Other myocardial insufficiency
429 Ill-defined heart disease
(430-438) Cerebrovascular disease
430 Subarachnoid haemorrhage
431 Cerebral haemorrhage
432 Occlusion of precerebral arteries
433 Cerebral thrombosis

434 Cerebral embolism
435 Transient cerebral ischaemia
436 Acute but ill-defined cerebrovascular disease
437 Generalised ischaemic cerebrovascular disease
438 Other and ill-defined cerebrovascular disease
(440-448) Diseases of arteries, arterioles and capillaries
440 Arteriosclerosis
441 Aortic aneurysm (non-syphilitic)
442 Other aneurysm
443 Other peripheral vascular disease
444 Arterial embolism and thrombosis
445 Gangrene
446 Polyarteritis nodosa and allied conditions
447 Other diseases of arteries and arterioles
448 Diseases of capillaries
(450-458) Diseases of veins and lymphatics, and other diseases of circulatory system
450 Pulmonary embolism and infarction
451 Phlebitis and thrombophlebitis
452 Portal vein thrombosis
453 Other venous embolism and thrombosis
454 Varicose veins of lower extremities
455 Haemorrhoids
456 Varicose veins of other sites
457 Noninfective disease of lymphatic channels
458 Other diseases of circulatory system

ICD-9
390 Rheumatic fever without mention of heart involvement
391 Rheumatic fever with heart involvement
392 Rheumatic chorea
(393-398) Chronic rheumatic heart disease
393 Chronic rheumatic pericarditis
394 Diseases of mitral valve
395 Diseases of aortic valve
396 Diseases of mitral and aortic valves
397 Diseases of other endocardial structures
398 Other rheumatic heart disease
(401-405) Hypertensive disease
401 Essential hypertension
402 Hypertensive heart disease
403 Hypertensive renal disease
404 Hypertensive heart and renal disease

405 Secondary hypertension
(410-414) Ischaemic heart disease
410 Acute myocardial infarction
411 Other acute and subacute forms of ischaemic heart disease
412 Old myocardial infarction
413 Angina pectoris
414 Other forms of chronic ischaemic heart disease
(415-417) Diseases of pulmonary circulation
415 Acute pulmonary heart disease
416 Chronic pulmonary heart disease
417 Other diseases of pulmonary circulation
(420-429) Other forms of heart disease
420 Acute pericarditis
421 Acute and subacute endocarditis
422 Acute myocarditis
423 Other diseases of pericardium
424 Other diseases of endocardium
425 Cardiomyopathy
426 Conduction disorders
427 Cardiac dysrhythmias
428 Heart failure
429 Ill-defined descriptions and complications of heart disease
(430-438) Cerebrovascular disease
430 Subarachnoid haemorrhage
431 Intracerebral haemorrhage
432 Other and unspecified intracranial haemorrhage
433 Occlusion and stenosis of precerebral arteries
434 Occlusion of cerebral arteries
435 Transient cerebral ischaemia
436 Acute but ill-defined cerebrovascular disease
437 Other and ill-defined cerebrovascular disease
438 Late effects of cerebrovascular disease

ICD-10
(I00-I02) Acute rheumatic fever
I00 Rheumatic fever without mention of heart involvement
I01 Rheumatic fever with heart involvement
I02 Rheumatic chorea
(I05-I09) Chronic rheumatic heart diseases
I05 Rheumatic mitral valve diseases
I06 Rheumatic aortic valve diseases
I07 Rheumatic tricuspid valve diseases

I08 Multiple valve diseases
I09 Other rheumatic heart diseases
(I10-I15) Hypertensive diseases
I10 Essential (primary) hypertension
I11 Hypertensive heart disease
I12 Hypertensive renal disease
I13 Hypertensive heart and renal disease
I15 Secondary hypertension
(I20-I25) Ischaemic heart diseases
I20 Angina pectoris
I21 Acute myocardial infarction
I22 Subsequent myocardial infarction
I23 Certain current complications following acute myocardial infarction
I24 Other acute ischaemic heart diseases
I25 Chronic ischaemic heart disease
(I26-I28) Pulmonary heart disease and diseases of pulmonary circulation
I26 Pulmonary embolism
I27 Other pulmonary heart diseases
I28 Other diseases of pulmonary vessels
(I30-I52) Other forms of heart disease
I30 Acute pericarditis
I31 Other diseases of pericardium
I32 Pericarditis in diseases classified elsewhere
I33 Acute and subacute endocarditis
I34 Nonrheumatic mitral valve disorders
I35 Nonrheumatic aortic valve disorders
I36 Nonrheumatic tricuspid valve disorders
I37 Pulmonary valve disorders
I38 Endocarditis, valve unspecified
I39 Endocarditis and heart valve disorders in diseases classified elsewhere
I40 Acute myocarditis
I41 Myocarditis in diseases classified elsewhere
I42 Cardiomyopathy
I43 Cardiomyopathy in diseases classified elsewhere
I44 Atrioventricular and left bundle-branch block
I45 Other conduction disorders
I46 Cardiac arrest
I47 Paroxysmal tachycardia
I48 Atrial fibrillation and flutter
I49 Other cardiac arrhythmias
I50 Heart failure
I51 Complications and ill-defined descriptions of heart disease

I52 Other heart disorders in diseases classified elsewhere
(I60-I69) Cerebrovascular diseases
I60 Subarachnoid haemorrhage
I61 Intracerebral haemorrhage
I62 Other nontraumatic intracranial haemorrhage
I63 Cerebral infarction
I64 Stroke, not specified as haemorrhage or infarction
I65 Occlusion and stenosis of precerebral arteries, not resulting in cerebral infarction
I66 Occlusion and stenosis of cerebral arteries, not resulting in cerebral infarction
I67 Other cerebrovascular diseases
I68 Cerebrovascular disorders in diseases classified elsewhere
I69 Sequelae of cerebrovascular disease
(I70-I79) Diseases of arteries, arterioles and capillaries
I70 Atherosclerosis
I71 Aortic aneurysm and dissection
I72 Other aneurysms
I73 Other peripheral vascular diseases
I74 Arterial embolism and thrombosis
I77 Other disorders of arteries and arterioles
I78 Diseases of capillaries
I79 Disorders of arteries, arterioles and capillaries in diseases classified elsewhere
(I80-I89) Diseases of veins, lymphatic vessels and lymph nodes, not elsewhere classified
I80 Phlebitis and thrombophlebitis
I81 Portal vein thrombosis
I82 Other venous embolism and thrombosis
I83 Varicose veins of lower extremities
I84 Haemorrhoids
I85 Oesophageal varices
I86 Varicose veins of other sites
I87 Other disorders of veins
I88 Nonspecific lymphadenitis
I89 Other non-infective disorders of lymphatic vessels and lymph nodes
(I95-I99) Other and unspecified disorders of the circulatory system
I95 Hypotension
I97 Postprocedural disorders of circulatory system, not elsewhere classified
I98 Other disorders of circulatory system in diseases classified elsewhere
I99 Other and unspecified disorders of circulatory system

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