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**COORDINATORE Prof. Persio Dello Sbarba**

# Vegetarian vs Mediterranean diet for prevention of cardiovascular disease


**Dottorando**
Dott.ssa Monica Dinu

**Tutore**
Prof. Francesco Sofi

**Coordinatore**
Prof. Persio Dello Sbarba


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# ABSTRACT

**Background:** Nutrition is able to alter the cardiovascular health of the general population. Actually, the optimal dietary strategy for cardiovascular prevention is yet far to be defined. Mediterranean and vegetarian diets are those reporting the greatest grade of evidence, but no experimental studies comparing these two dietary patterns are available.

**Aim:** The aim of my PhD study was to implement a randomized, open, crossover clinical trial in a population of omnivorous individuals living in a low-risk European country for cardiovascular disease, in order to compare the effects of a 3-month period of vegetarian and Mediterranean diets on reducing body weight, fat mass and various cardiovascular risk parameters.

**Methods:** We randomly assigned to overweight omnivores with a low-to-moderate cardiovascular risk profile a vegetarian diet compared to a Mediterranean diet. The outcomes were changes of body weight, body composition and risk parameters of cardiovascular disease from baseline. Anthropometric measurements and blood analyses were performed at both the beginning and end of each dietary intervention.

**Results:** One hundred-eighteen subjects (mean age: 51.1, females: 78%) were enrolled. The total participation rate at the end of the study was 84.7%. No significant difference between the vegetarian and Mediterranean diets was found, as both interventions produced equally effective results. In particular, a significant body weight reduction of 1.9 kg and 1.8 kg, with a significant BMI reduction of -0.6 kg/m$^2$ and -0.7 kg/m$^2$, and a significant fat mass reduction of 1.2 kg and 1.5 kg were reported in the vegetarian and Mediterranean diet groups, respectively. Significant differences between the two interventions were obtained for LDL-cholesterol and vitamin B12

levels, which significantly decreased by 5.44% and 5.06% only in the vegetarian diet group, and for triglyceride levels, which significantly decreased by 5.91% only in the Mediterranean diet group. Finally, there was no significant difference between dietary interventions in oxidative stress markers and inflammatory cytokines, except for interleukin-17, which improved only in the Mediterranean diet group. Overall, the vegetarian diet resulted in a reduction of 8 out of 13 cytokines with 6 that reached the statistical significance, whereas the Mediterranean diet determined a reduction of 11 out of 13 pro- and anti-inflammatory cytokines, with 7 that reached the statistical significance. Forty-six participants during the vegetarian diet period and 35 during the Mediterranean diet period reached the target values for at least one cardiovascular risk factor.

**Conclusions:** Both vegetarian and Mediterranean diets were effective in reducing body weight, BMI, and fat mass, with no significant differences between them. However, the vegetarian diet resulted more effective in reducing LDL-cholesterol levels, whereas the Mediterranean diet determined a significant reduction in triglyceride levels. These findings could be important for improving the awareness of the general population towards prevention-based interventions aimed at reducing cardiovascular disease.

# 1. INTRODUCTION

Nutrition is able to substantially alter the health status of the general population *(GBD 2013 Risk Factors Collaborators et al., 2015)*. During the past decades, a rapid expansion in the number of relevant scientific fields and, in particular, in the amount of population-based epidemiological evidence has clearly demonstrated the role of diet in preventing and controlling morbidity and premature mortality resulting from chronic diseases. In industrialized countries, the most important association between diet and health is certainly the relationship with cardiovascular disease, the leading cause of death and disability worldwide.

A vast abundance of literature demonstrated that both incidence and mortality from cardiovascular disease can be easily prevented with adequate dietary choices and correct lifestyles, such as moderate physical activity, abstinence from tobacco smoking and from harmful use of alcohol[1]. The ability to identify with certainty, therefore, the relationship between diet and cardiovascular disease appears to be a key element in the implementation of specific primary prevention strategies *(Oliveira et al., 2015)*.

Recent studies have focused their interest on the impact of a whole dietary approach rather than on isolating single nutrients on the occurrence of cardiovascular disease; it is now recognized that analyses of single nutrients ignore the important and complex interactions between components of a diet *Jacobs et al., 2007; Chahoud et al., 2004)*. However, the optimal dietary strategy for the prevention of cardiovascular disease has not been found *(WHO, 2007; Rees et al., 2013)*. Several models of diet have been imposed on public attention, but those that got the most interest are certainly Mediterranean and vegetarian diets *(Ha et al., 2015)*. These dietary patterns seem to exert protective effects on blood pressure, lipid profiles, cardiovascular diseases and metabolic parameters.

[1]http://www.health.gov/dietaryguidelines/2015-scientific-report/

The term Mediterranean diet has been widely used to describe the traditional dietary habits of people living in the South of Italy and in the Island of Crete, Greece, during the 1960s. This dietary pattern was characterized by plentiful plant-derived foods (fruits, vegetables, breads, other forms of cereals, beans, nuts, and seeds), olive oil as the principal source of fat, moderate amounts of dairy products (mainly cheese and yogurt), low-to-moderate amounts of fish and meat and a moderate consumption of red wine, usually during meals. Since the first data from the Seven Countries' Study, several studies in different populations have established a beneficial role for the main components of the Mediterranean diet on the occurrence of cardiovascular and chronic degenerative diseases *(Keys et al., 1986)*. A recent umbrella review conducted by our group have reported, in both meta-analyses of prospective cohort studies and randomized controlled trials, a robust evidence (supported by a p value < 0.001, a large simple size, and not a considerable heterogeneity between studies) for a greater adherence to the Mediterranean diet and a reduced risk of overall mortality, cardiovascular diseases, coronary heart disease, myocardial infarction, overall cancer incidence, neurodegenerative diseases and diabetes *(Dinu et al., 2017a)*.

Vegetarian diet is a dietary profile characterized by abstention from consuming meat and meat products, poultry, seafood and flesh from any other animal. Over the last decades, some case-control and cohort prospective studies reported beneficial effects of such dietary profile on the occurrence of cardiovascular and neoplastic disease. Recently, we conducted a systematic review with meta-analysis on over than 120,000 vegetarians, by showing that adherence to a vegetarian dietary pattern helps determining, among case-control studies, lower levels of the most important risk factor for chronic disease, along with a reduced risk of occurrence for ischemic heart disease (-25%) when cohort prospective studies were taken into account *(Dinu et al., 2017b)*.

Nevertheless, medical literature in this field still has some unresolved aspects that require further insights. In particular, most of the evidence that showed beneficial effects of vegetarian diet came from observational studies, or investigated the vegetarian diet in countries at high risk

for cardiovascular disease (e.g., United States), or were conducted among vegetarians, with all the biases related to the fact that they are also health-conscious subjects, and therefore not quite similar to the general population *(Kwok et al., 2014)*. Moreover, only few and limited randomized dietary intervention studies that investigated the beneficial effects of a vegetarian diet in clinically healthy omnivorous subjects have been conducted *(Prescott et al., 1988; Kestin et al., 1989; Sciarrone et al., 1993; Burke et al., 2007)*. Therefore, it is currently unclear whether the health benefits of this dietary pattern can be attributed to the absence of meat in the diet, to the increased consumption of particular food components, to the pattern of foods eaten within the vegetarian diet, or to other healthy lifestyle components often associated with vegetarianism.

# 2. CARDIOVASCULAR DISEASE

## 2.1. Definition and pathogenesis

Cardiovascular diseases are a group of disorders of the heart and blood vessels, including coronary heart disease, cerebrovascular disease and peripheral arterial disease. The underlying pathological process that leads to coronary heart disease and cerebrovascular disease is atherosclerosis, an inflammatory process affecting medium- and large-sized blood vessels throughout the cardiovascular system, characterized by fatty degeneration and vessel stiffening.

The atherosclerotic process is initiated when cardiovascular risk factors, through a chemical, mechanical or immunological insult, activate and/or injury the endothelium, thus contributing to endothelial dysfunction and fragmentation. Activated endothelial cells express vascular adhesion molecules including selectins, vascular cell adhesion molecule (VCAM)-1, and intercellular adhesion molecule1 (ICAM-1), which determine the recruitment of monocytes and T-cells, and the subsequent release of monocyte chemo-attractant protein-1 (MCP-1) by leukocytes.

In response to signals generated within the early plaque, monocytes adhere to the endothelium and then migrate through the endothelium and basement membrane by elaborating enzymes that degrade the connective tissue matrix. Recruited monocytes, which are transformed in macrophages, release additional cytokines and continue to migrate through the endothelial surface into media of the vessel. This process is further enhanced by the local release of monocyte-colony stimulating factor (M-CSF), which causes monocytic proliferation. The local activation of monocytes leads to both cytokine-mediated progression of atherosclerosis, and oxidation of low-density lipoprotein (LDL), particularly atherogenic and chemotactic for monocyte-macrophages.

The movement of LDL from the blood into the vessel wall starts the lipid deposition; in fact, once within the media, LDL may become oxidized through action of free radicals or direct

activity of leukocytes, or it may be taken up by macrophages, which became less mobile, thereby promoting the accumulation of these lipid-laden cells in the intima. The binding between macrophages and oxidized LDL occurs via a family of novel receptors known as scavenger receptors, which recognize LDL only after it has been oxidized.

Unregulated uptake of atherogenic lipoproteins by macrophages leads to the generation of foam cells, which are laden with lipid. Foam cells retain their metabolic activity by promoting the recruitment and proliferation of smooth muscle cells (which in turn elaborate additional locally active cytokines and inflammatory mediators), further LDL oxidation, recruitment of additional monocyte/foam cells and additional impairment of endothelial function. The accumulation of foam cells leads to the formation of fatty streaks, which are often present in the aorta of children, the coronary arteries of adolescents, and other peripheral vessels of young adults. Although they cause no clinical pathology, fatty streaks are widely considered to be the initial lesion leading to the development of complex atherosclerotic lesions.

Vascular smooth muscle cells - either recruited from the media into the intima or proliferating within the intima - contribute to the atherosclerotic process by secreting large amounts of extracellular-matrix components, such as collagen. The presence of these increases the retention and aggregation of atherogenic lipoproteins. In addition to monocytes, recruited T cells help to perpetuate a state of chronic inflammation. As the plaque grows, compensatory remodelling takes place, such that the size of the lumen is preserved while its overall diameter increases. Foam cells eventually die, resulting in the release of cellular debris and crystalline cholesterol. In addition, smooth muscle cells form a fibrous cap beneath the endothelium, and this walls off the plaque from the blood. This process contributes to the formation of a necrotic core within the plaque and further promotes the recruitment of inflammatory cells (Figure 2.1).

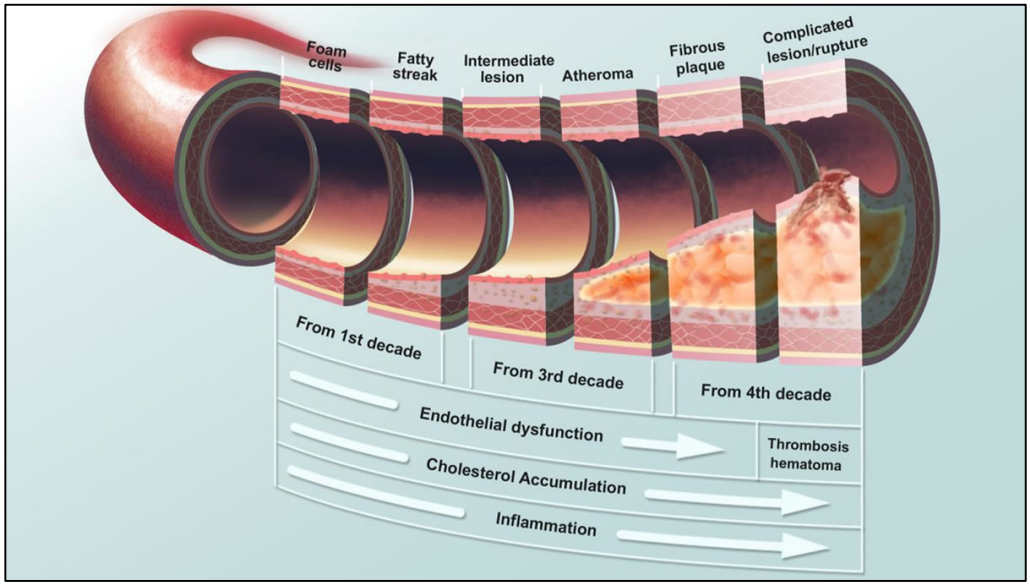


**Figure 2.1.** The process of atherogenesis (Source: *Health 2013; 5: 19-33*)

This non-obstructive plaque can impair or erode the endothelium, resulting in the exposure of thrombogenic material, including tissue factor, and the formation of a thrombus in the lumen. If the thrombus is large enough, it blocks the artery, which causes an acute coronary syndrome or myocardial infarction *(Libby et al., 2001)*. Ultimately, if the plaque does not rupture and the lesion continues to grow, the lesion can encroach on the lumen by resulting in clinically obstructive disease. Atherosclerosis can also occur in the arteries of the neck, kidneys, thighs, and arms, causing kidney failure or gangrene and amputation.

## 2.2. Epidemiology

Cardiovascular disease is the leading cause of mortality and morbidity worldwide. An estimated 17.9 million people died from cardiovascular disease in 2015, up from 12.3 million in 1990, representing 32.1% of all global deaths *(GBD 2015 Mortality and Causes of Death, Collaborators et al., 2015)*. In particular, an estimated 7.4 million were due to coronary heart

disease and 6.7 million were due to stroke. More than 3 millions of these deaths occurred before the age of 60 and could have largely been prevented.

Cardiovascular disease is also a major cause of loss of disability-adjusted life years (DALYs) - a composite of years of potential life lost due to premature death and years of productive life lost due to disability. In particular, cardiovascular disease is responsible for 10% of DALYs lost in low- and middle-income countries and 18% of DALYs lost in high-income countries. The worldwide burden of cardiovascular diseases is expected to increase to more than 23.6 million by 2030.

Examination of cardiovascular disease mortality trends across countries reveals considerable variability in the shape and magnitude of cardiovascular disease epidemics. From the 1930s to the 1950s, the rate of cardiovascular disease increased in high-income countries, but during this period, the rates were low in middle- and low-income countries *(Walker et al., 2004)*. Since the mid-1970s, the rate of death from cardiovascular diseases has declined markedly in several high-income countries, owing to reductions in risk factors and improved management of cardiovascular disease *(O'Flaherty et al., 2013)* (Figure 2.2).

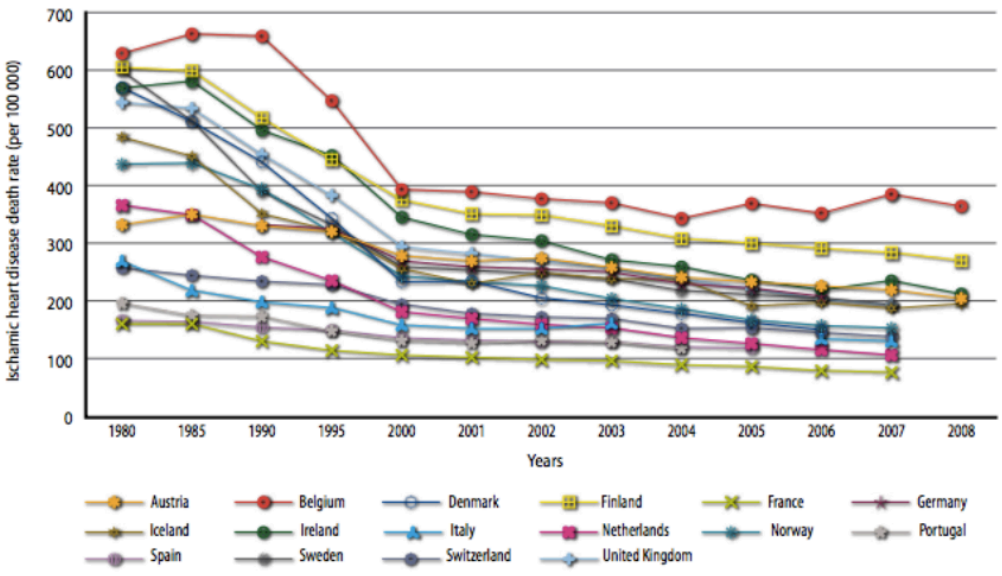


**Figure 2.2**. Trends in CVD mortality rates (age standardized) in developed countries (Source: *WHO/WHF/WSO 2011 Global atlas on CVD prevention and control)*

By contrast, the incidence of cardiovascular disease has been increasing in some low-income and middle-income countries, with 80% of the global burden estimated to occur in these countries (Yusuf et al., 2014) (Figure 2.3). Currently, the percentage of premature deaths from cardiovascular diseases ranges from 4% in high-income countries to 42% in low-income countries, leading to growing inequalities in the occurrence and outcome of cardiovascular diseases between countries and populations.

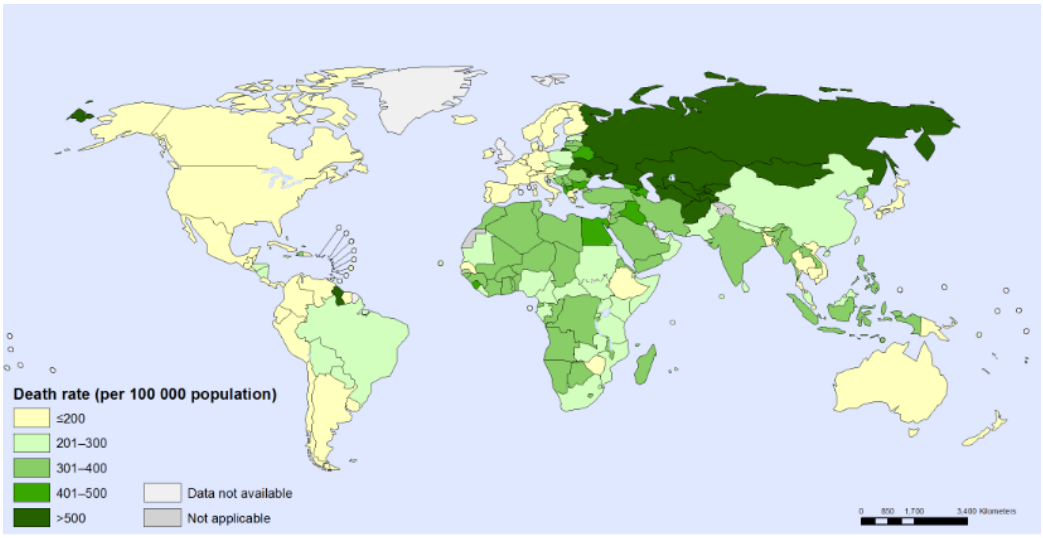


**Figure 2.3**. Age-standardized deaths due to cardiovascular disease (rate per 100,000), *WHO 2012*

Cardiovascular disease is also the leading cause of mortality in Europe, where they cause just over 3.9 million deaths a year (45% of all deaths) *(Wilkins et al., 2017)*. In men, cardiovascular disease accounts for 1.8 million deaths (40% of all deaths), while in women is responsible for 2.1 million deaths (49% of all deaths). The main forms of cardiovascular diseases are ischaemic heart disease and stroke.

Comparing the cardiovascular disease mortality burden across individual European countries reveals substantial variation, with a higher burden typically found in Central and Eastern European countries compared to that in Northern, Southern and Western countries. The proportion of all deaths ranges from 23% in France to 60% in Bulgaria among men, while

in women, the burden ranges from 25% in Denmark to 70% in Bulgaria *(Wilkins et al., 2017)*. Importantly, the same report states that dietary factors make the largest contribution to the risk of cardiovascular disease mortality and cardiovascular disease DALYs at the population level.

Over the past 25 years, deaths and disabilities from cardiovascular disease also determined a significant increase of hospital discharge rates. In Europe, overall cardiovascular disease is estimated to cost €210 billion a year, out of which around 53% (€111 billion) is due to health care costs, 26% (€54 billion) to productivity losses and 21% (€45 billion) to the informal care of people with cardiovascular disease.

## 2.3. Risk factors and prevention

Several epidemiological studies were implemented in the last century to unravel the causes of cardiovascular disease *(Doyle et al., 1957; Dawber et al., 1957; Keys et al., 1963)*. In 1932, Raab described the relationship between diet and coronary heart disease in different regions *(Raab et al., 1932)*, and in 1953 an association between cholesterol levels and coronary heart disease mortality was reported in various populations *(Keys at al., 1953)*. However, the expression "risk factor" was coined by the Framingham Heart Study, a pivotal cohort study initiated in 1948 by the USA Public Health Service to study the epidemiology and risk factors for cardiovascular disease *(Levy et al., 1988)*.

Nowadays, a risk factor is defined as a measurable element or characteristic that is causally associated with an increased rate of a disease and that is an independent and significant predictor of the risk of presenting a disease. Four years after the beginning of the Framingham study, investigators identified high cholesterol and high blood pressure as important factors in the development of cardiovascular disease. In the following years, Framingham and other epidemiological studies contributed to the identification of other risk factors that are now considered to be classical risk factors for cardiovascular disease (Figure 2.4). They have

subsequently been found to apply in different populations across the world, in both men and women and at all ages *(van den Hopgen et al., 2000)*.

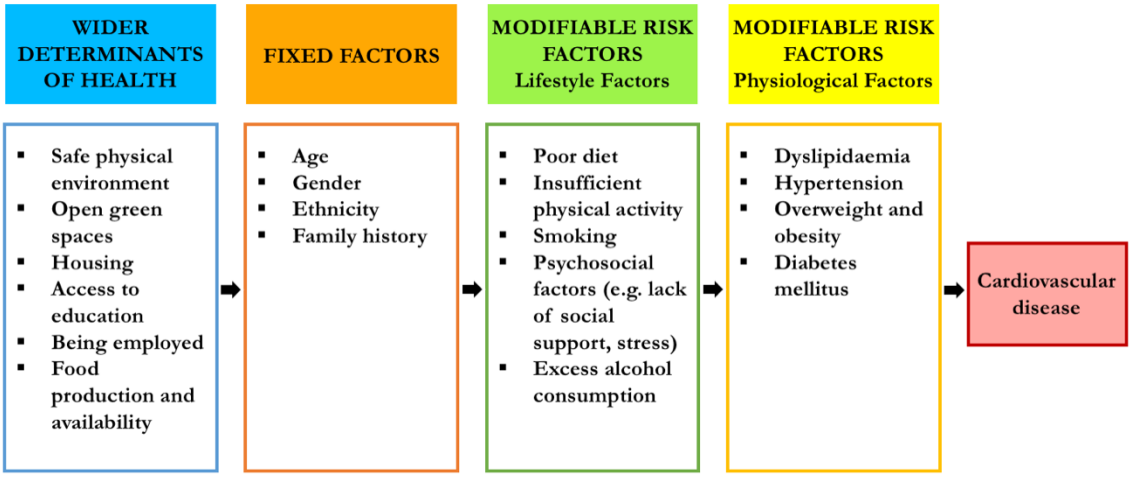


**Figure 2.4**. Risk factors for primary prevention of cardiovascular disease

While fixed risk factors are non-modifiable and cannot be changed (e.g. increased risk if a first degree blood relative has had coronary heart disease or stroke before the age of 55 years for male relative and 65 years for female relatives), making changes to lifestyle significantly reduces the risk of premature cardiovascular disease. Yusuf et al calculated a population attributable risk for first myocardial infarction of 90.4% associated with a combination of nine modifiable risk factors (smoking, fruit and vegetable consumption, physical activity, alcohol consumption, psychosocial factors, hypertension, dyslipidaemia, obesity and diabetes), indicating that a very high proportion of cardiovascular disease could be prevented if these risk factors were eliminated *(Yusuf et al., 2004)*.

**- *Diet:*** An unhealthy diet is an important modifiable risk factor that influences cardiovascular health either through an effect on risk factors such as cholesterol, blood pressure, body weight and diabetes mellitus, or through an effect independent of these risk factors[2]. High consumption of dietary fat, especially trans and saturated fats, increases the risk of atherosclerosis, while diets

[2]European Heart Network. Diet, Physical Activity and Cardiovascular Disease Prevention in Europe. Brussels, Belguim: European Heart Network, 2011.

high in sodium increase the risk of hypertension. Consumption of fruits, vegetables and dietary fibre meanwhile have a protective effect. In line with this evidence, the World Health Organization recommends eating at least 400 grams (five portions) or fruit and vegetables a day, consuming no more than 5 grams of salt each day, limiting dietary sugar intake and dietary fat intake to 10% and 30% of total energy intake respectively, and balancing total energy intake with energy expenditure[3]. The important role of nutrition as a determinant of cardiovascular disease, and in cardiovascular disease prevention, will be discussed further in the next chapter.

**- *Physical activity:*** Physical activity and aerobic exercise training are suggested by guidelines as a very important non-pharmacological tool for primary and secondary cardiovascular prevention *(Piepoli et al., 2016)*. Indeed, participation in regular physical activity and/or aerobic exercise training is associated with a reduction in cardiovascular disease prevalence and mortality by 20–30% in a dose–response fashion, while a sedentary lifestyle increases the risk of cardiovascular disease by increasing the risk of hypertension, high triglycerides, low HDL cholesterol, diabetes and obesity *(Lee et al., 2012)*. The World Health Organization recommends that adults undertake at least 150 minutes of moderate intensity aerobic physical activity per week, at least 75 minutes of vigorous intensity aerobic activity, or a combination of moderate and vigorous activities. In older adults aged 65 years and above, the World Health Organization additionally recommends participation in muscle strengthening activities on at least two days a week, while for children and adolescents, they recommend at least 60 minutes of moderate to vigorous intensity physical activity per day[4].

**- *Smoking habit:*** Changing smoking behaviour is the most cost-effective strategy for cardiovascular disease prevention; the 10-year fatal cardiovascular risk is approximately doubled in smokers *(Piepoli et al., 2016)*. The risks associated with smoking show a dose–response

[3]http://www.who.int/nutrition/publications/nutrientrequirements/healthydiet_factsheet394.pdf. Healthy Diet Factsheet. World Health Organization.
[4]http://apps.who.int/iris/bitstream/10665/44399/1/9789241599979_eng.pdf. Global recommendations on physical activity for health. World Health Organization, 2010.

relationship with no lower limit for deleterious effects[5]. On the other hand, the benefits of smoking cessation have a large evidence base, and are consistent over gender, duration of follow-up, study site and time period, with the risk of cardiovascular disease approaching (but never equalling) the risk of never smokers within 10–15 years. Significant morbidity reductions occur within the first 6 months *(Stead et al., 2008)*.

**- *Psychosocial factors***: Low socio-economic status, lack of social support, stress at work and in family life, depression, anxiety, hostility, and the type D personality contribute both to the risk of developing cardiovascular disease and the worsening of clinical course and prognosis of cardiovascular disease. These factors act as barriers to treatment adherence and efforts to improve lifestyle, as well as to promoting health and wellbeing in patients and populations. Multiple prospective studies have shown that men and women with low socio-economic status, defined as low educational level, low income, holding a low-status job, or living in a poor residential area, have an increased all-cause as well as cardiovascular disease mortality risk *(Stringhini et al., 2010; Alter et al., 2014; Rahimi et al., 2007)*. Recent systematic reviews confirm that people who are isolated or disconnected from others are at increased risk of dying prematurely from cardiovascular disease. Similarly, lack of social support leads to decreased survival and poorer prognosis among people with clinical manifestations of cardiovascular disease *(Barth et al., 2010)*. Work-related stress as well as conflicts or long-term stressful conditions in family life have also been shown to increase cardiovascular disease risk (Eaker et al., 2007; *Kivimaki et al., 2012; Kivimaki et al., 2015)*.

**- *Excess alcohol consumption:*** While low alcohol consumption is associated with lower coronary mortality *(Holmes et al., 2014)*, excessive alcohol intake is associated with increased

[5]http://www.surgeongeneral.gov/library/tobaccosmoke/index.html. Centers for Disease Control and Prevention. How Tobacco Smoke Causes Disease: The Biology and Behavioural Basis for Smoking-attributable Disease 2010. A Report of the Surgeon General.

cardiovascular mortality[6]. Alcohol ranks as the second-leading cause of DALYs lost in high-income countries. Both level and pattern of alcohol use has an effect on cardiovascular disease risk: drinking 3 or more alcoholic beverages per day is associated with elevated cardiovascular disease risk, while binge drinking is associated with a higher risk of sudden death and stroke (*Britton et al., 2000)*. Maximum cardiovascular benefit occurs at 1-2 standard drinks a day in men (10-20 g alcohol) and up to 1 a day in women (10 g alcohol).

***- Dyslipidaemia:*** Increased plasma cholesterol and LDL cholesterol are among the main risk factors for cardiovascular disease, while hypertriglyceridaemia and low HDL cholesterol are independent risk factors. The evidence that reducing plasma LDL cholesterol reduces cardiovascular disease risk is unequivocal; every 1.0 mmol/L reduction in LDL cholesterol is associated with a corresponding 20–25% reduction in cardiovascular disease mortality and non-fatal myocardial infarction *(Cholesterol Treatment Trialists' Collaboration et al., 2012)*. Trials have confirmed that lowering LDL cholesterol to ≤70 mg/dL is associated with the lowest risk of recurrent cardiovascular disease events in secondary prevention populations *(Baigent et al., 2010)*. Therefore, for very high-risk subjects, the target LDL cholesterol level should be ≤70 mg/dL or a ≥50% reduction from baseline LDL cholesterol. As regards hypertriglyceridemia, fasting triglycerides ≥150 mg/dL continue to be considered a marker of increased risk, but there are no randomized trials to provide sufficient evidence to derive target levels. HDL cholesterol is also a risk factor and is recommended to be used for cardiovascular disease risk estimation, but is not recommended as a target for treatment. Although recent Mendelian randomization studies cast doubt on the causal role of HDL cholesterol in cardiovascular disease *(Voight et al., 2012)*, an HDL cholesterol level >40 mg/dL in men and >45 mg/dL in women may be regarded as a marker of increased risk.

***- Hypertension:*** High blood pressure is a leading risk factor for disease burden globally, accounting for 9.4 million deaths and 7% of global DALYs in 2010 *Lim et al., 2012)*. Overall, the

[6]http://apps.who.int/iris/bitstream/10665/112736/1/9789240692763_eng.pdf. World Health Organization. Global status report on alcohol and health.

prevalence of hypertension is 30-45% in adult persons ≥18 years of age, with a steep increase with ageing. The risk of death from cardiovascular disease increases progressively and linearly from blood pressure levels as low as 115 mmHg systolic and 75 mmHg diastolic upwards, although for absolute risk the curves flatten in the lower blood pressure ranges *(Lewington et al., 2002)*. Unhealthy diet, physical inactivity and obesity all increase the risk of hypertension, while high blood pressure can be effectively lowered in response to lifestyle changes such as weight loss, increased physical activity and reduced salt and alcohol intake. Based on current data, it may be prudent to recommend lowering systolic/diastolic blood pressure to values within the range 130-139/80–85 mmHg, and possibly close to the lower values in this range, in all hypertensive patients.

**- *Overweight and obesity*:** Overweight and obesity are key risk factors for cardiovascular disease, resulting from a positive energy balance caused by energy consumption relative to energy expenditure through physical activity. As well as increasing the risk of cardiovascular disease directly, obesity is a risk factor for high blood pressure, dyslipidaemia, insulin resistance, systemic inflammation, prothrombotic state and the development of diabetes mellitus *(Piepoli et al., 2016)*. To achieve optimal health, the median BMI for adult populations should be in the range of 21-23 kg/m$^2$, while the goal for individuals should be to maintain a BMI in the range 18.5-24.9 kg/m$^2$. Diet, exercise, and behaviour modifications are the mainstay therapies for overweight and obesity.

**- *Diabetes mellitus*:** People with diabetes mellitus present a two-fold risk of occurring cardiovascular disease *(Emerging Risk Factors Collaboration et al., 2010)*. High blood sugar causes damage to blood vessels, thus increasing the risk of cardiovascular disease directly, as well as exacerbating the effects of other risk factors such as raised blood pressure, raised cholesterol levels, smoking and obesity. Clear reductions have occurred in cardiovascular disease death rates in diabetes mellitus consistent with better management of risk factors - blood pressure, lipid

control, glycaemia and HbA1c. Except for glucose management, prevention of cardiovascular disease follows the same general principles as for people without diabetes mellitus. Achieving low blood pressure levels and low LDL and total cholesterol concentrations is particularly important.

The importance of primary prevention has been greatly emphasized in recent years. Evidence obtained from applying the IMPACT model suggested that primary prevention may account for 2 to 4 times more of the mortality reduction than secondary prevention *(Young et al., 2010; Unal et al., 2005)*. Accordingly, Gemmell et al. estimated that meeting government targets for cardiovascular risk factors through primary prevention could prevent more events than increasing treatment levels in secondary prevention *(Gemmel et al., 2006)*. Therefore, strategies to prevent cardiovascular disease have global significance and should be a top priority for individuals and for the healthcare systems. With regard to individuals, prevention ideally starts during childhood and lasts until the end of life. Even exposure to risk factors before birth may influence the lifetime risk *(Forsen et al., 1999)*.

As of healthcare systems, there are two approaches towards prevention of cardiovascular disease: the population strategy in which risk across a whole population is reduced, and the high-risk strategy where preventative action is focused on high-risk individuals *(Rose et al., 1985)*. More specifically, the population strategy aims at reducing the cardiovascular disease incidence at the population level through lifestyle and environmental changes targeted at the population at large. This strategy is primarily achieved by establishing ad-hoc policies and community interventions. Examples include measures to ban smoking and reduce the salt content of food. The advantage is that it may bring large benefits to the population although it may offer little to the individual.

In the high-risk approach, preventive measures are aimed at reducing risk factor levels in those at the highest risk, either individuals without cardiovascular disease at the upper part of the total cardiovascular risk distribution or those with established cardiovascular disease. An example of a high-risk intervention in cardiovascular disease prevention is a screening

programme that identifies people who are at high risk of developing cardiovascular disease and offers them interventions (e.g. statins and lifestyle advice) to reduce their risk. Although individuals targeted in this strategy are more likely to benefit from the preventive interventions, the impact on the population level is limited, because people at such high risk are few. In addition, cardiovascular disease prevention in people at low risk such as young people might result in substantial benefit, since behavioural patterns are framed during childhood and adolescence and are maintained or even promoted by the individual's social environment as an adult.

For a long time, the population strategy has been considered to be more cost-effective than the high-risk approach but since the introduction of highly effective lipid lowering drugs, improvement in smoking cessation programmes and lower costs of antihypertensive drugs, the effectiveness of the high risk approach has increased *(Manuel et al., 2006)*. There is now consensus that the largest preventive effect is achieved when these are combined, obtaining the reduction of the overall level of risk for the whole population (Figure 2.5).

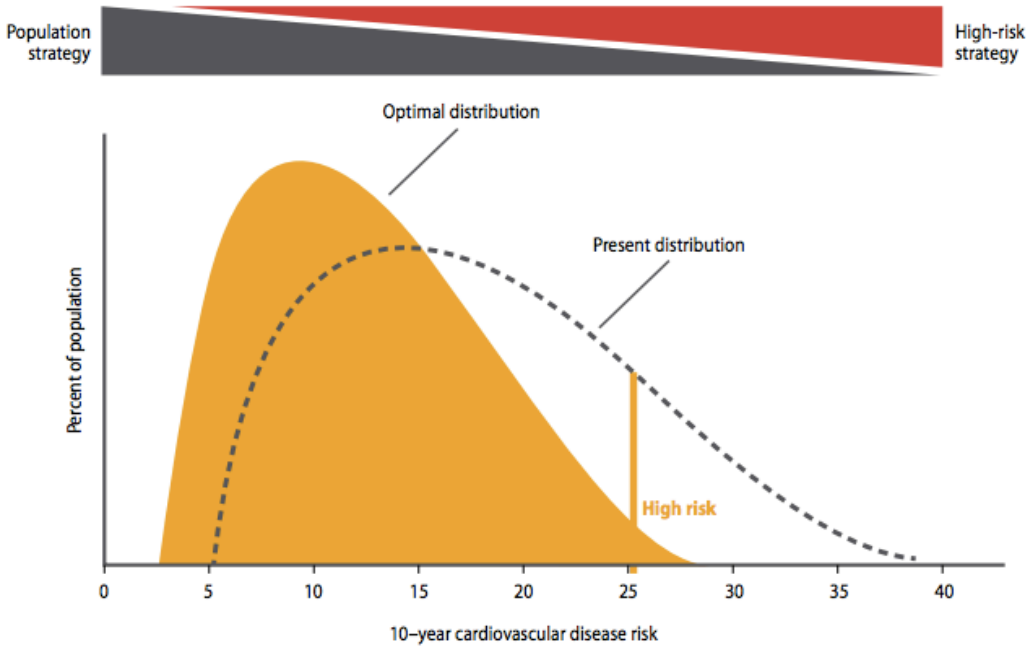


**Figure 2.5**. The ideal prevention strategy – shifting the population health outcome curve to the left (Source: *WHO/WHF/WSO 2011 Global atlas on CVD prevention and control)*

# 3. NUTRITION AND CARDIOVASCULAR DISEASE

## 3.1. Characteristics of a heart-healthy diet

Of particular relevance to this thesis is the well-established association between nutrition and cardiovascular disease, which has been studied intensively for nearly a century. The first evidence of a possible relationship between dietary habits and occurrence of diseases dates back to the years following the World War II, when significant variations in the incidence of cardiovascular diseases and other major non-communicable diseases were observed in studies conducted in migrants that moved from countries with a favourable dietary profile to a country with an unfavourable and industrialized profile. Subsequently, many studies investigated the influence of diet and nutrition on the pathogenesis of the disease states through using analytical, ecologic and epidemiological approaches.

A balanced diet, in terms of both quantity and quality, represents a key factor for the optimal prevention of cardiovascular disease, by reducing the risk of occurrence of the first cardiovascular event and improving the prognosis after the first event *(Freeman et al., 2017)*. On the other hand, an unhealthy diet is an important risk factor; evidence from the Global Burden of Disease study suggests that the proportion of disability-adjusted life-years from ischemic heart disease attributable to poor diet may exceed those attributable to tobacco smoking, alcohol or physical inactivity *(Lim et al., 2012)*.

The result from the INTERHEART study *(Yusuf et al., 2004)*, a large case-control study that investigated risk factors for myocardial infarction within 52 countries including non-developed, developing and industrialized countries, confirmed that diet is one of the most important risk factors for the occurrence of myocardial infarction, independently from all the other parameters. The authors evaluated numerous risk factors, including smoking, history of hypertension and diabetes mellitus, waist to-hip ratio, dietary patterns, physical activity, alcohol consumption, the ratio of apolipoprotein (apo) B to apo A-I, and psychosocial factors.

Collectively, 9 risk factors - smoking, fruit and vegetable consumption, physical activity, alcohol consumption, psychosocial factors, hypertension, dyslipidaemia, obesity and diabetes - accounted for 90% of the risk of a first occurrence of acute myocardial infarction in men and 94% of the risk in women. Noticeably, 8 of 9 of these factors are strongly influenced and modifiable by diet.

Dietary factors influence many or even all of the cardiovascular and metabolic risk pathways in a number of ways (Figure 3.1).

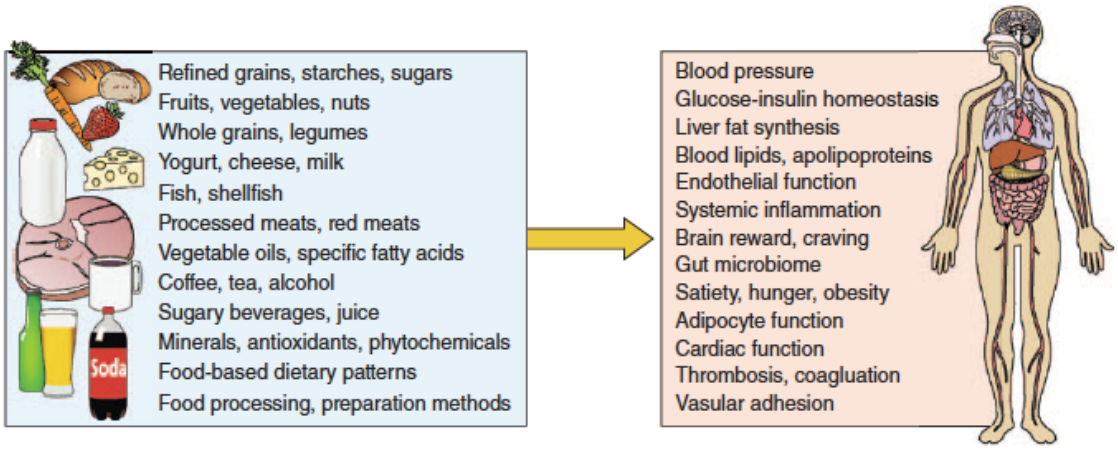


**Figure 3.1**. Diet and cardiovascular and metabolic risk - pathways and mechanisms

(Source: *Circulation 2016; 133: 187-225*)

While previous recommendations for a healthy diet were heavily single-nutrient-focused, modern evidence underlines that specific foods and overall diet patterns are most relevant. Common characteristics of dietary patterns associated with positive health outcomes include higher intake of vegetables, fruits, whole grains, low- or non-fat dairy, fish, legumes, and nuts; moderate intake of alcohol (among adults); lower consumption of red and processed meat, and low intake of sugar-sweetened foods and drinks, and refined grains.

- ***Fruits and Vegetables*** - Consumption of fruits and vegetables is associated with a reduction in the risk of cardiovascular disease as well as many of the most important neoplasms *(Aune et al.,*

*2017)*. In addition, fruits and vegetables represent the main source of fiber and an important source of vitamins (especially B-group) and antioxidant. In randomized controlled trials fruit and vegetable consumption has shown substantial improvements in various risk factors such as blood pressure, plasma lipid levels, pro-inflammatory parameters, endothelial function, weight control, and circulating insulin levels *(Hartely et al., 2013)*. The benefits do not appear to be reproducible with equivalent amounts of minerals, vitamins and with fiber supplements, nor are dependent on the composition of macronutrients in the diet *(Schwingshackl et al., 2017)*. This evidence suggests that benefits might be derived from:

- a more complex set of micronutrients, phytochemicals, and fiber found in fruits and vegetables
- potentially enhanced bioavailability of these nutrients in their natural state
- replacement of less healthful foods in the diet

In long-term observational studies, the consumption of 200g/day of fruit and vegetables has been associated with lower incidence of coronary heart disease (-8%), stroke (-16%) and cardiovascular disease (-8%) *(Aune et al., 2017)*.

**- *Whole Grains*** - Although no single accepted definition of whole grain exists, whole grains generally comprise bran, germ, and endosperm from the natural cereal. Bran contains soluble and insoluble dietary fiber, B vitamins, minerals, flavonoids, and tocopherols; germ contains numerous fatty acids, antioxidants, and phytochemicals. Endosperm provides largely starch (carbohydrate polysaccharides) and storage proteins. The type and extent of processing appear to modify the health effects of grain and carbohydrate consumption. For example, removal of bran and germ reduces dietary fiber that has important benefits, including lowering of blood pressure and cholesterol levels; increases bioavailability and rapidity of digestion of remaining endosperm, which increases glycaemic responses; and eliminates minerals, micronutrients, and other phytochemicals that may have additional independent health benefits. The consumption of whole grain cereals with a high fiber content has a double benefit on health. First, fiber

consumption from cereal products is associated with lower risk of cardiovascular risk factors and heart disease *(Hartley et al., 2016; Wu et al., 2015)*. Secondly, increased dietary fiber consumption reduce the risk of developing type 2 diabetes, facilitate weight control and helps prevent constipation (*Dahl et al., 2015)*. In randomized clinical trials it has been demonstrated that whole grain consumption improves insulin resistance and endothelial function and reduces inflammatory parameters *(Seal et al., 2015)*. Whole grain consumption reduces LDL cholesterol, without reducing HDL cholesterol or triggering triglycerides *(Hollænder et al., 2015)*. Consistently with the physiological benefits, a greater consumption of whole grains is associated with a lower incidence of cardiovascular disease, coronary heart disease and diabetes mellitus *(Aune et al., 2016a)*. Similar to fruits and vegetables, health effects of whole grains may result from synergistic effects of multiple constituents that are unlikely to be matched by supplemental fiber alone, added bran, or isolated micronutrients.

**- *Fish*** - Fish and other seafood contain several healthful constituents, including specific proteins, unsaturated fats, vitamin D, selenium, and long-chain omega-3 polyunsaturated fatty acids (PUFAs), which include eicosapentaenoic acid (EPA; 20:5 omega-3) and docosahexaenoic acid (DHA; 22:6 omega-3). In humans, EPA and especially DHA are synthesized in low amounts (5%) from their plant-derived precursor, linolenic acid (18:3 omega-3). Thus, tissue levels of EPA plus DHA are strongly influenced by their direct dietary consumption. Average EPA plus DHA contents of different seafood species vary by 10-fold. Fatty (oily) fish such as anchovies, herring, farmed and wild salmon, sardines, trout, and white tuna tend to have the highest concentrations. In vitro and animal experiments suggest that fish oil has direct antiarrhythmic effects, but trials to establish direct antiarrhythmic effects in patients with preexisting arrhythmias have been inconsistent. In human experiments, fish oil is able to lower triglyceride levels, systolic and diastolic blood pressure, and resting heart rate *(Siscovick et al., 2017)*. Observational evidence and randomized clinical trials suggest that fish oil or fish consumption can reduce inflammation, improve endothelial function, normalize heart rate variability,

improve myocardial relaxation and efficiency, and, at high doses platelet aggregation *(Mozaffarian et al., 2011)*. Consistent with these physiological benefits, habitual consumption of fish is associated with a lower incidence of coronary heart disease and ischemic stroke, particularly heart failure. Consumption of 250 mg/d of EPA and DHA from fish is associated with a 36% reduction in mortality for cardiovascular disease. Overall, these findings are concordant with long-term observational studies of habitual fish intake (2-3 times/week) in generally healthy populations and with physiological benefits of fish or fish oil in intervention studies.

**- *Nuts:*** A decade of epidemiologic investigation and clinical studies has transformed the image of nuts from a fattening snack food to a wholesome and heart-healthy food. Nuts are high in fat, but most of the fat is monounsaturated and polyunsaturated. Numerous metabolic studies have found that a diet high in nuts (peanuts, walnuts, or almonds) significantly lowers LDL cholesterol and decreases the ratio of total to HDL cholesterol. Besides the favourable effects on blood lipids, nuts may protect against cardiovascular disease through other mechanisms: e.g. most nuts are rich in arginine (the precursor of nitric oxide), α-linolenic acid, magnesium, copper, folic acid, potassium, fiber, and vitamin E. A meta-analysis of prospective cohort studies has shown that daily consumption of 30 g of nuts reduces the risk of cardiovascular disease by 30% *(Luo et al., 2014)*. In line with these results, the meta-analysis by Aune et al. found that an intake of approximately 15–20 grams of nuts per day or 5–6 servings per week provides the maximum reduction of coronary heart disease, stroke, and cardiovascular disease risk *(Aune et al., 2016b)*. These findings support dietary recommendations to increase nut consumption to reduce cardiovascular disease risk and premature mortality in the general population.

**- *Legumes:*** Eating legumes as part of a healthy diet may help improving blood cholesterol, a leading cause of cardiovascular disease *(Bazzano et al., 2011)*, as well as body weight *(Kim et al., 2016)*. Legumes are the best source of vegetable protein and contain minerals and fiber. High

plant protein intake was inversely associated with cardiovascular mortality, especially in subjects with at least one unhealthy lifestyle factor *(Song et al., 2016)*. Results from the NHANES study clearly showed that legumes' consumption ≥4 times a week is associated with an 11% lower risk of cardiovascular disease and a 22% reduced risk of coronary heart disease compared to once per week *(Bazzano et al., 2001)*. A recent meta-analysis reported a 14%-reduction of ischemic heart disease risk for a consumption of legumes that reach 4 portions (100 g/serving) per week *(Afshin et al., 2014)*.

**- *Dairy Foods*** - Dairy products represent a class of foods with complex effects that vary by specific product type and with emerging mechanistic pathways that appear to include influences of fermentation and probiotics. On the basis of lower content of calories, saturated fatty acids, and cholesterol, most dietary guidelines and scientific organizations recommend low-fat or non-fat dairy consumption. Such recommendations largely derive from theoretical considerations about selected single nutrients (calcium, vitamin D, calories, saturated fat), rather than empirical evidence on health effects of the actual foods. In longitudinal studies evaluating habitual intakes of dairy foods, relationships with cardiovascular disease and diabetes mellitus do not consistently differ by fat content but appear more specific to food type: e.g. cheese, yogurt, milk, butter *(Hu et al., 2014; Qin et al., 2015)*. Interestingly, dairy fat itself may promote cardiometabolic health. In cohorts using objective blood biomarkers, greater dairy fat consumption is associated with a lower incidence of diabetes mellitus *(Kratz et al., 2013)* and coronary heart disease *(de Oliveira Otto et al., 2013)* and with mixed findings for stroke *(Yakob et al., 2014)*. It remains unclear whether such findings relate to health benefits of specific dairy fatty acids (e.g. branched-chain fatty acids, medium-chain saturated fats, specific ruminant trans fats), other lipid-soluble factors in dairy fat, other factors in high-fat dairy foods (e.g. production of vitamin K2 from fermentation of cheese), or unknown endogenous determinants of these blood biomarkers. Previous systematic reviews and meta-analyses have published different reports regarding the effect of dairy consumption on cardiovascular disease *(de Goede et al., 2016; Drouin-Chartier et al., 2016)*. Recently, the meta-

analysis by Gholami et al. showed a possible inverse association between total dairy intake and cardiovascular disease and stroke *(Gholami et al., 2017)*, while the meta-analysis by Guo et al suggested neutral associations of total, high and low-fat dairy, milk and yogurt with risk of all-cause mortality, coronary heart disease and cardiovascular disease *(Guo et al., 2017)*. A possible role of fermented dairy was found in cardiovascular disease prevention, but the result was driven by a single study. Although additional data are needed to more comprehensively examine the association between different dairy products and cardiovascular disease, the current science supports consuming more yogurt and possibly cheese.

**- *Meat and meat products:*** Diets rich in meat and derivatives have been associated with an increased risk of developing cardiovascular disease and stroke *(Wang et al., 2016; Yang et al., 2016)*. This association, particularly strong for red processed meat, may be explained by the harmful effect of several compounds in meat, such as sodium, nitrates, heme iron, as well as saturated fat and dietary cholesterol *(Etemadi et al., 2017)*. As current evidence suggests that each serving per day (50 g) of processed meat increase the cardiovascular mortality by 24%, it would be prudent to minimize or avoid processed meats such as sausage, salami, bacon and low-fat deli-meats (1 or less serving/week), and consume small amounts of unprocessed red meat (1 serving/week) *(Abete et al., 2014)*. Relatively few studies have been focused on poultry as a risk factor for cardiovascular disease, showing mixed results. In the context of a balanced diet, poultry consumption should be limited to 2-3 servings/week.

**- *Sugar-sweetened foods and drinks:*** Sugar has no nutritional value except for calorie intake and thus has negative health implications for people at risk of overweight. Added sugar and sugar-sweetened foods and drinks contribute to increase the cardio-metabolic risk including weight gain, diabetes, and metabolic syndrome, as well as cardiovascular disease outcomes *(Khan et al., 2016)*. The 2015 Dietary Guidelines Advisory Committee suggests the maximum

restriction of added sugars and sweetened foods and beverages[7], while the WHO recommends a maximum limit of 10% of energy from free sugar *(Mente et al., 2016)*. A further reduction to <5% per day would provide additional benefits.

Despite the spread of information on diet and dietary habits, progressive change in eating habits has been observed in recent years with a progressive worsening of nutritional quality in terms of prevention *(Imamura et al., 2015)*. Therefore, the main scientific associations have indicated some major recommendations to follow a healthy diet, especially for preventing cardiovascular disease (Table 3.1).

**Table 3.1.** Recommendations for preventing cardiovascular disease

| |
|---|
| • Reduce the calories |
| • Increase the consumption of fruit and vegetables |
| • Increase the consumption of legumes |
| • Increase the consumption of whole grain products |
| • Reduce the consumption of fresh and processed meat |
| • Replace saturated and trans fats with unsaturated fats |
| • Increase food sources of omega-3 fatty acids |
| • Limit the consumption of added sugar and sugar-sweetened beverages |
| • Limit sodium intake |

The identification of the best heart-healthy diet is still a challenging and highly relevant preventive health issue. Several models of diet have been imposed on public attention, but those that got the most interest are certainly Mediterranean and vegetarian diets.

[7]http://www.health.gov/dietaryguidelines/2015-scientific-report/. Dietary Guidelines Advisory Committee. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. 2015.

# 4. MEDITERRANEAN DIET

## 4.1. Characteristics

The Mediterranean diet is a typical dietary pattern traditionally followed by the people living in the countries bordering the Mediterranean Sea. This dietary pattern as described in the Mediterranean Diet pyramid *(Bach-Faig et al., 2011)* is generally characterized by a high consumption of plant foods (such as fruit, vegetables, legumes, nuts and seeds and cereals, preferably wholegrain); the seasonal choice of fresh and locally grown produce as far as possible; the presence of fruit as the main daily dessert and olive oil as the main source of dietary lipids; moderate consumption of dairy products (mainly cheese and yoghurt); low to moderate amounts of fish, poultry and eggs; consumption of red meat at a low frequency and in small amounts; and a moderate intake of wine during meals. Considered one of the healthiest dietary patterns for preventing diseases and maintaining the health status, the Mediterranean diet was also recognized by UNESCO as a cultural heritage of humanity.

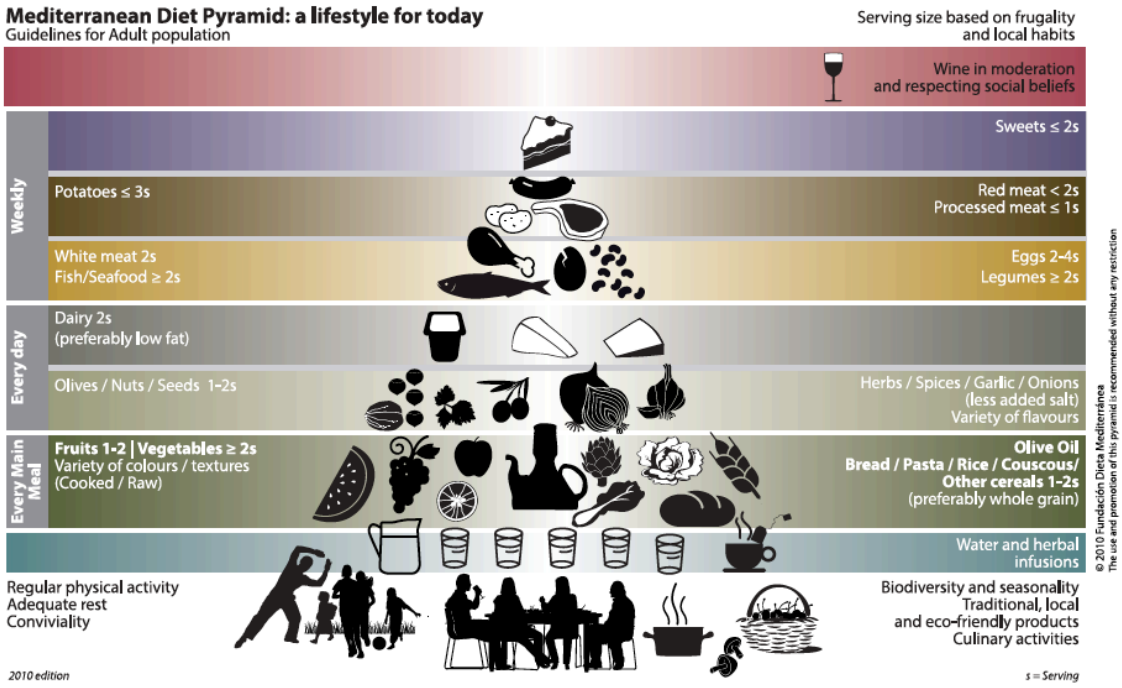


**Figure 3.2.** Mediterranean diet pyramid

## 4.2. Mediterranean diet and cardiovascular health

The first evidence about the positive effects of the Mediterranean diet has been supplied by the "Seven Countries' Study", the first epidemiological study aimed at investigating the possible association between diet and diseases, across different countries over an extended period of time *(Keys et al., 1986)*. In this study, designed by Ancel Keys at the beginning of the 1950s, dietary behaviour, lifestyles and health status of nearly 13,000 subjects aged between 40 to 59 years living in 7 different countries (Italy, Greece, the Netherlands, United States, Finland, Japan, former Yugoslavia) were analysed. At the end of the 25-year follow-up, about one half of death cases were due to coronary heart disease, with mortality rates remarkably differing in various countries *(Menotti et al., 1993)*. In particular, a lower mortality rate was recorded in Greece and in the South of Italy with 25 death cases every 1,000 inhabitants, whereas the highest mortality rate was recorded in Finland with 268 death cases every 1,000 inhabitants. The reduced incidence of cardiovascular disease reported by the Mediterranean countries during this study opened a new field of investigation to conduct epidemiological and clinical studies aimed at confirming these preliminary results.

Starting from 1995, when the first study by Trichopoulou et al showed an association between the grade of adherence to the Mediterranean diet and mortality *(Trichopoulou et al., 1995)*, several studies have reported an association between a greater adherence to Mediterranean diet and a favourable health status. In 2003, findings from the Greek cohort of the EPIC study reported that a 2-point increase in the adherence score to Mediterranean diet determined a reduction in total and coronary heart disease mortality *(Trichopoulou et al., 2003)*. Two years later, the same authors confirmed their data, by using a slightly modified diet-score in relation to cardiovascular diseases *(Trichopoulou et al., 2005)*.

These data were confirmed by two cohort studies carried out in Spain *(Martinez-Gonzalez et al., 2011; Buckland et al., 2009)*. In the first, on a sample of 13,609 adults (mean age: 38 years) who were followed for an average of 4.9 years, the adherence to the Mediterranean diet was associated with a reduced incidence of cardiovascular disease. Participants were evaluated by

using a frequency questionnaire and those who had followed the Mediterranean diet with higher adherence had a 59% reduction in the risk of cardiovascular disease compared to those who reported a lower adherence (RR: 0.41, 95%CI: 0.18-0.95). In particular, an increase of 2 points in the Mediterranean dietary score was associated with a 26% reduction in the onset of coronary artery disease (RR: 0.74, 95% CI: 0.55-0.99). In the second work, carried out on the cohort of 41,078 subjects aged between 29 and 69 years of the EPIC study, and followed for a mean follow-up of 10.4 years, a 40% reduction in cardiovascular events was observed in subjects who followed more closely the Mediterranean dietary pattern (RR: 0.60, 95% CI: 0.47-0.77), compared to those who reported a lower adherence.

A similar conclusion was also reached by the longitudinal study HALE, including the SENECA and FINE studies, both multicentre studies from different European countries, Mediterranean and non, of 2,239 elderly (>70 years) followed for a mean follow-up of 10 years *(Knoops et al., 2004)*. Adherence to a healthy lifestyle, considered as the combination of four major risk factors, such as adherence to the Mediterranean diet, moderate alcohol consumption, smoking cessation, and a regular physical activity, determined a lower risk of mortality from coronary heart disease by 39% (RR: 0.61, 95% CI: 0.43-0.88) and from cardiovascular disease by 29% (RR 0.71, 95% CI: 0.58-0.88).

The protective effect of the Mediterranean diet in relation to cardiovascular disease was also demonstrated in non-Mediterranean populations, such as in the U.S. Indeed, in two large cohort studies, adherence to Mediterranean diet was found to significantly modify the cardiovascular risk profile *(Mitrou et al., 2007; Fung et al., 2009)*. The first, the study by Mitrou et al, analyzed 214,284 people aged between 50 and 71 years for a follow-up period of about 10 years. The population of patients who had a greater adherence to the Mediterranean diet had a 22% reduction in cardiovascular mortality in men (RR: 0.78, 95% CI: 0.69-0.87) and 29% in women (RR: 0.81, 95% CI: 0.68-0.97). The second study, comprising a population of 74,486 women that attended the Nurses' Health Study and evaluated for 20 years of follow-up, confirmed this protective association with adherence to the Mediterranean diet. Women who

reported a higher adherence to the Mediterranean diet had a significant protection of 29% (RR 0.71, 95% CI: 0.62-0.82) against coronary heart disease, with a reduction in overall mortality from cardiovascular disease by approximately 40% (RR: 0.61, 95% CI: 0.49-0.76).

Studies have shown that adherence to the Mediterranean diet is also able to reduce the risk of ischemic stroke. In the aforementioned study of Fung et al., a greater adherence to the Mediterranean diet reduced by 13% (RR: 0.87, 95% CI: 0.73-1.02), the risk of experiencing an ischemic stroke in the population of women attending the Nurses' Health Study *(Fung et al., 2009)*. Similarly, a case-control study conducted in a population of 250 patients with ischemic stroke compared with 500 control subjects confirmed the potential protective effect of a greater adherence to the Mediterranean diet in relation to this disease (-12%) (RR: 0.88, 95% CI: 0.82-0.94) *(Kastorini et al., 2011a)*.

Similar results were reported in groups of patients already suffering from cardiovascular disease, therefore in secondary prevention. One of the most important work is the Lyon Diet Heart Study *(de Lorgeril et al., 1999)*, a clinical trial in which 605 patients with previous myocardial infarction were divided and assigned to an intervention group who followed a Mediterranean diet and to a control group that followed a so-called prudent diet. After a median follow-up period of 27 months, the rate of coronary events and mortality in the intervention group was reduced by 73% and 70% respectively, thus concluding that the use of a Mediterranean diet enriched with one gram of α-linolenic acid, was able to reduce quickly, markedly and long-lasting the risk of death, nonfatal myocardial re-infarction, and other clinical end-points.

Another important study in secondary prevention is that one conducted on 11,323 patients with prior myocardial infarction from 172 Italian hospitals. This study, called the GISSI-Prevention, showed that the Mediterranean diet is able to halve the risk of mortality and cardiovascular recurrences in this type of selected high-risk patients, regardless of medication and lifestyle (RR: 0.51, 95% CI: 0.44-0.59) *(Barzi et al., 2003)*. In addition, two studies that included subjects from the same cohort of the EPIC study, with a previous diagnosis of

myocardial infarction published in 2005 and 2007 confirmed the association between adherence to the Mediterranean diet and survival *(Trichopoulou et al., 2005; Trichopoulou et al., 2007)*. In the first study, the Greek cohort of patients with prior coronary artery disease (n = 1,302) were followed for a mean follow-up of approximately 4 years, by reporting a reduction in cardiovascular mortality by 27% (RR: 0.73, 95% CI: 0.58-0.93). In the latest study the population included the total population of persons from the countries of the EPIC study with a previous diagnosis of myocardial infarction (n = 2,671) followed by a median follow-up of 7 years. In this analysis, an increase of two units in the score of adherence to the Mediterranean diet was associated with an 18%-reduction in mortality (RR: 0.82, 95% CI: 0.73-0.93).

In the last years, the increasing number of studies conducted to investigate the effects of the Mediterranean diet on health have exponentially increased the level and the quality of the scientific evidence. Importantly, findings from observational studies have been confirmed in large randomized controlled trials such as the Prevenciòn con Dieta Mediterranea study (PREDIMED) *(Estruch et al., 2013)*. This clinical trial, conducted on almost 7,500 subjects, tested the efficacy of two Mediterranean diets, one supplemented with extra-virgin olive oil and other with nuts, as compared with a low-fat control diet, on primary cardiovascular prevention. After a median follow-up of 4.8 years, the Mediterranean diet supplemented with either extra-virgin olive oil or nuts resulted in an absolute risk reduction of approximately 30% among high risk persons who were initially free of cardiovascular disease. Similarly, a recent meta-analysis found that subjects in the highest quantile of adherence to the Mediterranean diet have a 24% lower incidence and mortality from cardiovascular disease compared to those least adherent (*Grosso et al., 2017)*. A significant reduction of risk was also found for stroke (-24%), myocardial infarction (-33%) and coronary heart disease (-28%).

The positive effect of the Mediterranean diet on cardiovascular health likely related to greater effectiveness of this dietary pattern in lowering main cardiovascular risk factors such as body mass index, lipid and glycaemic profile, as well as inflammatory parameters when

compared with other diets. Indeed, several meta-analyses demonstrated a beneficial effect of a Mediterranean diet on lipid variables, including total and LDL-cholesterol *(Nordmann et al., 2011; Rees et al., 2013; Garcia et al., 2016)*. In addition, a recent study reported that, even though the Mediterranean diet was not able to raise HDL levels, it enhanced the cardio protective capacity of HDL cholesterol by improving HDL oxidative status and composition *(Hernànez et al., 2017)*. Suggestive evidence also supports an effect on improving glycaemic control and insulin sensitivity *(Kastorini et al., 2011b; Huo et al., 2015; Nordmann et al., 2011)*. These parameters, linked to sugars and saturated fatty acids consumption, and therefore to excess energy intake and increased adiposity, are independent risk factors for cardiovascular disease *(Evert et al., 2014)*. Recent findings on the beneficial effects of the Mediterranean on inflammatory parameters have been also obtained *(Evert et al., 2014; Martínez-González et al., 2015)*. Inflammation has a key role at all the stages of the cardiovascular disease and is crucial in the development and rupture of the atheromatous plaque. Mediterranean diet has been found to determine a significant reduction of circulating levels of C-reactive protein, interleukin-6 and cell adhesion molecules, determining a down-regulation of cellular and humoral inflammatory pathways related to atherosclerosis *(Casas et al., 2017)*.

Given the high number of meta-analyses performed on the Mediterranean diet so far, we recently performed an umbrella review of the evidence across meta-analyses of observational studies and randomized controlled trials *(Dinu et al., 2017b)*. Our aim was to summarize the evidence and evaluate the validity of the association between the adherence to the Mediterranean diet and health outcomes. Umbrella reviews or overviews of existing systematic reviews and/or meta-analyses are relatively new study designs that help providing a comprehensive and systematic examination of the scientific literature available for a specific research topic. We identified 13 meta-analyses of observational studies and 16 meta-analyses of randomized controlled trials investigating the association between the adherence to the Mediterranean diet and 37 different health outcomes, for a total population of over than

12,800,000 subjects. A robust evidence, supported by a p value <0.001, a large simple size, and not a considerable heterogeneity between studies, for a greater adherence to the Mediterranean diet and a reduced the risk of overall mortality, cardiovascular diseases, coronary heart disease, myocardial infarction, overall cancer incidence, neurodegenerative diseases and diabetes was found (Figure 3.3). In addition, a reduced risk for cardiovascular disease incidence and mortality, myocardial infarction and coronary heart disease was evidenced in both meta-analyses of prospective cohort studies and intervention studies.

# 5. VEGETARIAN DIET

## 5.1. Characteristics

The vegetarian diet is characterized by the exclusion of meat, fish, or fowl or products containing these foods. The eating patterns of vegetarians may vary considerably. The lacto-ovo-vegetarian eating pattern is based on grains, vegetables, fruits, legumes, seeds, nuts, dairy products, and eggs but excludes meat, fish, and fowl. The vegan eating pattern is similar to the lacto-ovo-vegetarian pattern, with the additional exclusion of dairy and other animal products. Even within these patterns, considerable variation may exist in the extent to which animal products are avoided.

Of relevance to this thesis is the lacto-ovo-vegetarian diet, which is the most common type of vegetarianism and is actually supported by an increasing number of studies that show a protective effect against cardiovascular disease. According to the Academy of Nutrition and Dietetics, an appropriately planned vegetarian diet is healthful, nutritionally adequate, and may provide health benefits for the prevention and treatment of certain diseases; it is appropriate for all stages of the life cycle, including pregnancy, lactation, infancy, childhood, adolescence, older adulthood, and for athletes *(Melina et al., 2016)*.

The key to a healthy vegetarian diet - like any diet - is to include a variety of foods. Loma Linda University School of Public Health, Department of Nutrition developed The Vegetarian Food Pyramid in 1997 (Figure 3.3). The 5 major plant-based food groups (whole grains, legumes, vegetables, fruits, nuts and seeds) form the trapezoid-shaped lower portion of the pyramid. Optional food groups (vegetable oils, dairy and sweets) form the triangle-shaped top portion of the pyramid.

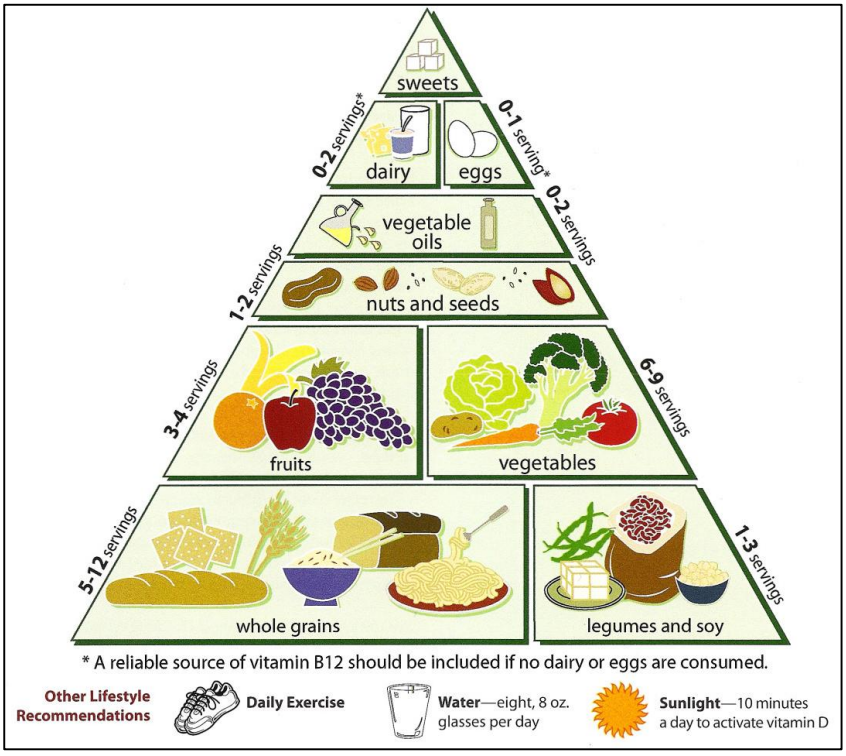


**Figure 3.3.** The Vegetarian Food Pyramid

The number of people who, in recent years, began to follow a food profile characterized by the absence of animal flesh such as meat and fish, has progressively and continuously increased, especially among industrialized countries *(Leitzmann et al., 2014)*. Reasons to adopt this dietary profile are different, ranging from ethical motivations, religious beliefs, environmental and cultural issues, to health-related aspects. In the latter case they arise from the desire to lose weight, in tackling obesity, improving physical fitness and/or in reducing the risk of acquiring certain diseases.

## 5.2. Vegetarian diet and cardiovascular health

Although the number of studies that investigated the effects of a vegetarian diet on health has increased, the amount of evidence is still limited with respect to the evidence for the Mediterranean diet. Seven prospective cohort studies evaluated the relationship between the vegetarian diet and cardiovascular health so far. Overall, the studies included almost 240,000 participants, half of which were vegetarians at the time of the evaluation.

The first study, established in 1960, was the Adventist Mortality Study (AMS) that recruited 27,529 members of the Seventh-day Adventist church in California *(Key et al., 1999)*. Seventh-day Adventists have been increasingly subject to epidemiological studies since they tend to be more homogenous in many aspects of lifestyle and more heterogeneous in eating habits than the general population. Adventists have a lower risk of chronic diseases than the general population, and many researchers hypothesize that this is due to their diet and lifestyle habits *(Kwok et al., 2014)*. In fact, in addition to the high prevalence of vegetarianism, such populations generally present low rates of smoking, consume little amount of alcohol, and possibly have a high level of physical activity. In this study, after 5.6 years of follow-up, the death rate ratios for heart disease and for cerebrovascular disease were significantly lower ($p<0.05$) in vegetarians (HR: 0.74; 95% CI: 0.63, 0.88 and HR: 0.65; 95% CI: 0.48, 0.87 respectively), compared with the regular meat eaters *(Key et al., 1999)*.

Other two studies on Adventists, the Adventist Health Study-1 (AHS-1) and the Adventist Health Study-2 (AHS-2), involving 34,000 and 96,000 subjects respectively, were conducted over the last 40 years *(Fraser et al., 1999; Orlich et al., 2014)*. In the AHS-1, vegetarians showed a lower mortality rate for heart disease (HR: 0.62; 95% CI: 0.53, 0.73 when compared to omnivores. Contrariwise, in omnivores, a greater incidence of obesity, hypertension and type 2 diabetes mellitus has been observed. The AHS-2, which started in 2002, is still in progress. Preliminary data show that vegetarians tend to have less cardiovascular risk factors (e.g. lower BMI, lower cholesterol and blood glucose levels and a lower prevalence of hypertension, overweight and obesity, type 2 diabetes mellitus and metabolic syndrome). In men, a significant reduction of cardiovascular events has been observed (RR: 0.71; 95% CI: 0.57, 0.90).

While Adventist cohorts suggested a potentially relationship between the vegetarian diet and the reduction of cardiovascular disease, the other cohorts demonstrated a modest effect. For example, the Health Food Shopper Study (HFSS) highlighted not significant differences in cardiovascular diseases (Death Rate Ratio DRR: 0.95; 95% CI: 0.84, 1.07), heart disease (DRR: 0.85; 95% CI: 0.71, 1.01), and cerebrovascular disease (DRR: 0.99; 95% CI: 0.79, 1.24)

between vegetarians and omnivores *(Appleby et al., 2002)*. This cohort, comprising nearly 11,000 subjects, was recruited between 1973 and 1979 from the customers of health food shops, members of vegetarian societies, and readers of relevant magazines. Subjects living in the United Kingdom joined the study by completing a short questionnaire.

Similar results were observed in the Heidelberg Study, a German prospective study that has followed for 21 years - from 1984 to 2005 - a total of 1,225 vegetarians and 679 "health-conscious" omnivores *(Chang-Claude et al., 2005)*. Study subjects were individuals following a vegetarian or "healthy" lifestyle who were initially recruited in 1976 from readers of relevant vegetarian magazines in Germany using a short questionnaire. The study participants were generally highly educated, about 40% having received >12 years of schooling. Vegetarians had lower median BMI than omnivores (20.7 versus 21.3) and reported more often a high level of physical activity. Smoking rates were very low, with only 3.9% of vegetarians and 8.0% of omnivores being smokers at the time of recruitment. The overall mortality rate for cardiovascular diseases (RR: 0.95; 95% CI: 0.62, 1.12) and heart disease (RR: 0.70; 95% CI: 0.41, 1.18) between the two groups did not show significant differences but it was reduced compared to the general population.

The Oxford Vegetarian Study (OVS), a prospective study conducted on 11,040 British participants recruited between 1980 and 1984, found lower BMI and lower levels of total and LDL-cholesterol in vegetarians, but significant differences between vegetarians and omnivores have not been highlighted in terms of cardiovascular outcomes *(Appleby et al., 2002)*. Finally, the Oxford component of the EPIC study monitored 65,000 men and women living in the United Kingdom, recruited between 1993 and 1999 through collaboration with family physicians and through the postal service. After 11.6 years of follow-up vegetarians had a 32% (HR: 0.68; 95% CI: 0.58, 0.81) lower risk of heart disease than omnivores. This result was only slightly attenuated after adjustment for BMI and did not differ by sex, age, BMI, smoking, or the presence of cardiovascular risk factors *(Crowe et al., 2013)*. According to the authors, the risk difference was probably mediated through the effect of the vegetarian diet on cholesterol levels

and blood pressure. However, a recent study that analysed pooled mortality data from the EPIC-Oxford cohort and the OVS, for a total of over 60,000 subjects comprising 20,324 vegetarians, found no difference in mortality from circulatory disease (HR: 1.12; 95% CI: 0.96, 1.30), heart disease (HR: 1.04; 95% CI: 0.83, 1.30) and cerebrovascular disease (HR: 1.15; 95% CI: 0.88, 1.50) between vegetarians and omnivores *(Appleby et al., 2016)*.

With the aim of clarifying these controversial findings, we conducted a comprehensive systematic review with meta-analysis of observational studies (i.e. cross-sectional studies, case-control, nested case-control, or case-cohort design) that reported a measure of association between vegetarian diet, assessed by questionnaires, and risk factors for chronic degenerative diseases and incidence/mortality from cardiovascular disease *(Dinu et al., 2017a)*. Ten prospective cohort studies and 86 cross-sectional studies were included. The overall analysis among prospective cohort studies reported a significant 25%-reduced risk of incidence from heart disease in a population of over 65,000 vegetarians.

The most likely explanation for the finding of a lower risk of incident heart disease in vegetarians is that vegetarians have generally lower values of established risk factors for cardiovascular disease than omnivores, but why this finding is not replicated for heart disease mortality, as observed in the study by Appleby et al. (2016), is unclear. Possible explanation is the fact that incident, nonfatal heart disease may lead to the effective medical management of established risk factors for heart disease (e.g., drugs to treat non-HDL cholesterol and high blood pressure), lowering subsequent heart disease mortality and partially nullifying the differences between vegetarians and omnivores found for heart disease incidence. In addition, the heart disease risk factors that are affected by consuming a vegetarian diet are, proportionally, more strongly related to the risk of heart disease at younger ages *(Lewington et al., 2007)*. This might explain the larger difference in heart disease mortality between regular meat eaters and vegetarians before age 75 y than for mortality up to age 90 y *(Appleby et al., 2016)*.

In the same meta-analysis *(Dinu et al., 2017a)*, the overall analysis of cross-sectional studies reported significant reduced levels of BMI (-1.49 kg/m$^2$), serum total cholesterol (-28.16

mg/dL), LDL cholesterol (-21.27 mg/dL), HDL cholesterol (-2.72 mg/dL), serum triglycerides (-11.39 mg/dL), and blood glucose levels (-5.08 mg/dL) in vegetarians versus omnivores. Evidence that vegetarian diets effectively lower BMI, blood concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and non-high-density lipoprotein cholesterol came also from meta-analyses of randomized controlled trials *(Barnard et al., 2015; Wang et al., 2015; Huang et al., 2016)*.

In conclusion, the positive effect of the vegetarian diet on heart health is likely due to a greater variety and amount of plant foods. Abundant consumption of vegetable, fruit, legumes, unrefined cereals and nuts, essential components of the vegetarian dietary pattern, has consistently been associated with a lower risk of cardiovascular disease *(Freeman et al., 2017)*. The complex mixture of phytochemicals, highly present in this diet, possess potent antioxidant activity and show synergistic and additive effects. Non-dietary factors may also contribute towards the efficacy of a vegetarian diet, since vegetarians tend to be more conscious for the health aspects, slimmer, and in better health when compared with omnivores; for this reason, specific cohorts have been demonstrated to be not generalizable to the general population for the low prevalence of risk factors *(Kwok et al., 2014)*.

# 6. AIM OF THE STUDY

The aim of my PhD study was to implement a randomized, open, crossover clinical trial in a population of omnivorous individuals living in a low-risk European country for cardiovascular disease, in order to compare the effects of a 3-month period of vegetarian and Mediterranean diets on the following markers of risk of cardiovascular disease:

- weight, BMI, body composition
- lipid profile
- glycaemic profile
- oxidative stress markers
- inflammatory parameters

# 7. EXPERIMENTAL PROCEDURES

## 7.1. Study design and sample

The study was designed as a randomized, open, crossover dietary trial with two intervention periods each lasting 3 months, and was conducted at the Unit of Clinical Nutrition of the Careggi University Hospital, Florence, Italy.

The sample size was determined through an online calculator software[8], based on previous studies conducted to verify the effectiveness of vegetarian-like diets on type 2 diabetic patients *(Mishra et al., 2013)*. To obtain 80% power to detect an effect size between 1.25 and 2.1, at an alpha level of 0.05, we needed 2 groups with minimum 50 subjects in each group. The calculation of an adequate sample size was based on conservative estimates of a 10 to 25% dropout rate of subjects. The recruitment was done by trial personnel using advertisements in local media, newspapers, social media, sheets and official websites. Subjects were also recruited from a pre-existing database of participants and from friends or relatives of the Careggi University Hospital staff. Before enrolment, a written informed consent was obtained from each participant.

After a 2-week run-in period, used to assess the motivation, commitment and availability of participants, subjects were randomly divided into two groups, each assigned to either the group which received a vegetarian diet, or the group which received a Mediterranean diet. Following the first intervention phase, the participants crossed over in order to obtain the second intervention phase, as depicted in Figure 7.1. A washout period was not implemented in order to reduce the loss of simple size. There were five clinical evaluations of the study population: at baseline before treatment had started, 1.5 months after the beginning of the first dietary intervention, 3 months after the beginning of the first dietary intervention and at the time of

[8] http://www.powerandsamplesize.com

crossing over, 4.5 months from the beginning of the study and 1.5 from the crossing over, and finally 6 months after the start of the study and 3 months from the crossing over.

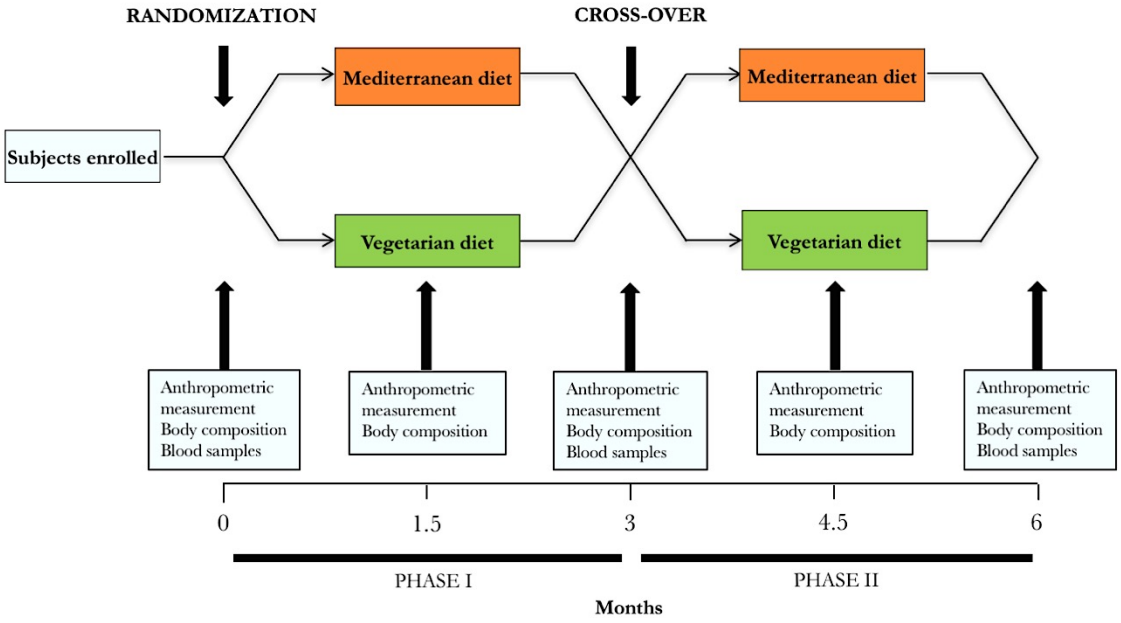


**Figure 7.1.** Organization of the intervention study

This design was implemented to allow comparison of vegetarian and Mediterranean diets within the same individual, in an effort to minimize attrition and to maximize participant interest and compliance by enabling each participant to experience both diet conditions. The study protocol was approved by the Ethic Committee (SPE 15.054) of the Tuscany Region, Careggi University Hospital, was registered at clinicaltrials.gov (identifier: NCT02641834), and adhered to the principles of the Declaration of Helsinki and the Data Protection Act.

## 7.2. Outcomes

Primary outcomes were changes in total weight, body mass index (BMI) and fat mass after 3-month intervention diet.

Secondary outcomes were changes in lipid profile [total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides], glycaemic profile [fasting glucose, insulin, HOMA-Index],

oxidative stress markers' [total antioxidant profile, parameters of lipid peroxidation] and inflammatory parameters' [circulating levels of inflammatory cytokines: IL-1ra (interleukin-1ra), IL-1 β (interleukin-1 beta), IL-2 (interleukin-2), IL-4 (interleukin-4), IL-6 (interleukin-6), IL-8 (interleukin-8), IL-10 (interleukin-10), IL-12 (interleukin-12), IL-18 (interleukin-17), IL-18 (interleukin-18), TNF-α (tumor necrosis factor alpha), IFN-γ (interferon-gamma), VEGF (vascular endothelial growth factor), MCP-1 (monocyte chemoattractant protein-1), IP-10 (IFN-γ-inducible protein 10)] after 3-month intervention diet.

## 7.3. Eligibility criteria

The inclusion criteria were as follows: clinically healthy subjects (age: 18-75 years) with a low-to-medium cardiovascular risk profile (1-5% according to the guidelines of the European Society of Cardiology)[9], determined by being overweight (Body Mass Index ≥25.0 kg/m$^2$) and by the concomitant presence of at least one the following criteria:

- Waist circumference > 88 cm (women) or >102 cm (men)
- Circulating levels of total cholesterol >190 mg/dL, not on drug treatment (measured no more than 3 months prior to the start of the study)
- Circulating levels of LDL cholesterol >115 mg/dL, not on drug treatment (measured no more than 3 months prior to the start of the study)
- Circulating levels of triglycerides >150 mg/dL, not on drug treatment (measured no more than 3 months prior to the start of the study)
- Circulating levels of fasting blood glucose >110 mg/dL but <126 mg/dL, not on drug treatment (measured no more than 3 months prior to the start of the study)

*Exclusion criteria* were presence of current serious illness or unstable condition that required physician supervision of diet or physical activity (e.g., recent myocardial infarction, chronic liver

[9] European Guidelines on cardiovascular disease prevention in clinical practice (version 2012)' The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts) * Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur Heart J 2012; 33: 1635-701.

disease, inflammatory bowel diseases); pregnancy or intention to become pregnant in the next 18 months*;* lactation*;* current or recent (past 6 months) participation in weight loss treatment program or use of weight loss medication*;* no regular intake of meat, fish, or poultry for the past 6 months.

## 7.4. Intervention diets

The vegetarian diet was characterized by abstinence to consume meat and meat products, poultry, fish and seafood, and flesh from any other animal. It included eggs and dairy products as well as all the other food groups. The Mediterranean diet was characterized by the consumption of all the food groups including meat and meat products, poultry, and fish. However, red meat was limited to once per week, poultry was limited to ≤3 times per week and fish was recommended 2-3 times per week.

We provided the participants with a detailed 1-week menu plan for each dietary intervention period, with all foods expressed in weight and/or volume measures, and a hand-out containing details on their assigned diet, including food groups that could be included and ones that needed to be avoid. The vegetarian menu plan included also recipes for preparing meals.

Vegetarian and Mediterranean diets were hypocaloric with respect to the energy requirements of subjects, but completely isocaloric between them, supplying 1,400 Kcal/day for women and 1,600 Kcal/day for men with a weight <90 kg at baseline, and 1,600 Kcal/day for women and 1,800 Kcal/day for men with a weight >90 kg at baseline. Both diets consisted of approximately 50-55% of energy from carbohydrate (primarily complex), 25-30% from total fat (≤7% of energy from saturated fat, <200 mg/day of cholesterol) and 15-20% from proteins.

Dietary profiles in terms of servings per week, calculated on the basis of the portion sizes recommended by the Italian Recommended Dietary Allowances[10], are shown in Table 7.1.

[10] Livelli di Assunzione di Riferimento di Nutrienti e Energia per la popolazione Italiana (LARN). Società Italiana di Nutrizione Umana (SINU). IV revisione.

**Table 7.1.** Frequency of consumption of food groups in the two diets

| **Food group** | **Vegetarian diet** | **Mediterranean diet** |
|---|---|---|
| **Cereals** | 20.5 servings/week | 21.5 servings/week |
| *Pasta* | 4 servings/week | 4 servings/week |
| *Rice* | 1 serving/week | 1 serving/week |
| *Polenta* | 1 serving/week | - |
| *Pizza* | 1 serving/week | 1 serving/week |
| *Wholegrain bread* | 6.5 servings/week | 8.5 servings/week |
| *Breakfast cereals* | 2 servings/week | 2 servings/week |
| *Rusks* | 3 servings/week | 3 servings/week |
| *Biscuits* | 1 serving/week | 1 serving/week |
| *Croissant* | 1 serving/week | 1 serving/week |
| **Vegetables** *(without potatoes)* | 15 servings/week | 14.5 servings/week |
| **Fruit** | 18 servings/week | 18 servings/week |
| **Nuts** | 2 servings/week | 1 serving/week |
| **Potatoes** | 1.5 serving/week | 1.5 serving/week |
| **Legumes** | 5 servings/week | 2.5 servings/week |
| **Eggs** | 2 servings/week | 1 serving/week |
| **Dairy products** | 21.5 servings/week | 18.5 servings/week |
| *Low fat milk* | 11 servings/week | 11 servings/week |
| *Low fat yoghurt* | 6 servings/week | 5 servings/week |
| *Cheese* | 4.5 servings/week | 2.5 servings/week |
| **Poultry** | - | 2 servings/week |
| **Red meat** | - | 1.5 serving/week |
| **Processed meat** | - | 1 serving/week |
| **Fish** | - | 2.5 servings/week |
| **Sweets** | 3.5 servings/week | 3.5 servings/week |
| **Olive oil** | 14 servings/week | 14 servings/week |

Servings/week are calculated according to the portion sizes recommended by the Italian Recommended Dietary Allowances (pasta: 80 g; rice: 80 g; polenta: 80 g; pizza: 200 g; wholegrain bread: 50 g; breakfast cereals: 30 g; rusks: 30 g; biscuits: 50 g; croissant: 50 g; vegetables: 200 g; fruit: 150 g; nuts: 30 g; potatoes: 200 g; legumes: 150 g; eggs: 50 g; low fat milk: 125 ml; low fat yoghurt: 125 g; cheese: 75 g; poultry: 100 g; red meat: 100 g; processed meat: 50 g; fish: 150 g; sweets: 20 g; olive oil: 10 ml)

There were no substantial differences in the frequency of servings per week for cereals, fruit and vegetables, potatoes, sweets and olive oil. On the other hand, the vegetarian diet reported a higher frequency of consumption per week for legumes (5 servings vs. 2.5 servings),

nuts (2 vs. 1), eggs (2 vs. 1) and dairy products (21.5 vs. 18.5) with respect to the Mediterranean diet. For both diets, alcoholic beverages were limited to 1 per day for women, and 2 per day for men. No meals or supplements were provided.

Interventions were delivered in a face-to-face individual counseling at the Clinical Nutrition Unit of Careggi University Hospital, Florence. We made no comment favouring one diet over the other or indicating our dietary habits. Participants were invited not to alter their lifestyle and exercise habits during the study, and no weight loss goal was given.

## 7.5. Data collection

Data collection and follow-up measurements were performed at the Clinical Nutrition Unit of Careggi University Hospital, Florence, Italy, by trial personnel. All subjects were examined between 6.30 a.m. and 9.30 a.m. after an overnight fasting period. Participants were asked not to undertake a strenuous physical activity during the day before the exam.

During the run-in period, subjects were asked to complete a 3-day (2 week-days and 1 weekend day) dietary record which was delivered to us at the first visit. Standardized baseline assessment for both groups included a questionnaire on demographic information, risk factors, and comorbidities. All subjects were asked to report the frequency (times per week), duration (months or years of exercise) and intensity of recreational and physical activities performed during the preceding year. The physical activity grade was derived for each subject based on frequency, type, and duration of the physical activity into qualitative terms such as absent or light (i.e. inactive or either occasional walking or recreational activity only), moderate (i.e. frequent recreational activities, regular walking for 30 minutes 3-5 times per week or sporting exercise at least once a week). The grade was not a measure of total time spent in physical activity but rather a relative qualitative measure of how much physical activity has been carried out.

*Anthropometric measurements and body composition*

Body weight and body composition were measured at each clinical evaluation. Weight and height were measured using a stadiometer. BMI was calculated as the weight (kg)/height ($m^2$). Patients were classified as overweight if their BMI was ≥25 kg/$m^2$ but <30 kg/$m^2$ and obese if their BMI was 30 kg/$m^2$ or more. Body composition was determined by a bioelectrical impedance analysis device (TANITA, model TBF-410).

*Blood samples*

Blood samples were collected at the beginning and at the end of each intervention phase. Blood samples were centrifuged at 3,000 rpm for 15 minutes, aliquoted to yield serum, and then stored at -80°C until analyses. The following biochemical measurements were evaluated: complete blood count, lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides), glucose profile (fasting glucose, insulin, HBA1c), liver function tests [aspartate aminotransferase, alanine transaminase, gamma-glutamyl transferase], renal function tests (uric acid), mineral profile (sodium, potassium, magnesium, calcium), iron metabolism (iron, ferritin), vitamin profile (vitamin $B_{12}$, folic acid), pro- and anti-inflammatory profile [circulating levels of inflammatory cytokines: IL-1ra (interleukin-1ra), IL-1 β (interleukin-1 beta), IL-2 (interleukin-2), IL-4 (interleukin-4), IL-6 (interleukin-6), IL-8 (interleukin-8), IL-10 (interleukin-10), IL-12 (interleukin-12), IL-18 (interleukin-17), IL-18 (interleukin-18), TNF-α (tumor necrosis factor alpha), IFN-γ (interferon-gamma), VEGF (vascular endothelial growth factor), MCP-1 (monocyte chemoattractant protein-1), IP-10 (IFN-γ-inducible protein 10)], as well as antioxidant profile [TBARs (ThioBarbituric Acid Reactive Substances), TAC (Total Antioxidant Capacity), L-ROS (Leukocytes–derived Reactive Oxygen Species), M-ROS (Monocytes-derived Reactive Oxygen Species), G-ROS (Granulocytes-derived Reactive Oxygen Species)].

## 7.6. Compliance and acceptability

Compliance with the intervention was critical to the success of this project and was achieved using behaviour change strategies including self-monitoring, and regular phone calls for dietary counselling. Vegetarian diet compliance was evaluated with an unannounced telephone call during which a 24-hour diet recall was administered, and through a modified version of the NHANES food questionnaire[11]. Adherence to the vegetarian diet was defined as the absence of any animal flesh reported by both 24-hour diet recall and food frequency questionnaire. Mediterranean diet compliance was evaluated at baseline and during the follow-up visits using the Mediterranean diet adherence score recently released and validated by our group *(Sofi et al., 2017)*. Subjects were considered adherent if they reported ≥10 points in a score ranging from 0 to 18.

## 7.7. Data management

Data were collected on an electronic database. Identifiable data were not recorded in the database. Other documents and participants were identified by a unique trial ID only. Hard copies of data sheets linking the participant identification number to the person's contact details were kept securely in a locked filing cabinet in a locked office, accessible only to key research team members. Given the limited objectives and its short-term nature, this trial was monitored by the protocol team and the local Institutional Review Board, without the use of a formal data monitoring committee. Data access was restricted to trained staff with unique password-protected accounts. No adverse events were reported.

[11] NHANES Food questionnaire
(available at https://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/tq_fpq_c.pdf)

## 7.8. Laboratory measurements

Venous blood samples were collected at baseline and at the end of each intervention phase in evacuated plastic tubes (Vacutainer, Beckton Dickinson, Plymouth, UK). Samples were centrifuged at 3,000 rpm for 15 minutes (4° C) and stored in aliquots at -80° C until further analyses. Total cholesterol and its subtypes, triglycerides, glucose, insulin, serum electrolytes, standard liver panel enzymes, mineral and vitamin profiles were measured according to conventional laboratory standard methods. To assess plasma oxidative stress profile, lipid peroxidation markers were estimated by using Thiobarbituric Acid Reactive Substances (TBARS) assay kit (Oxitek-ZeptoMetrix Corporation Buffalo, NY, US). Plasma total antioxidant capacity (TAC), which represents the overall antioxidant defense system, was measured using the Oxygen radical absorbance capacity (ORAC) *(Barygina et al., 2015)*. Reactive oxygen species' (ROS) production by leukocytes (lymphocytes, monocytes and granulocytes) was measured as previously reported *(Becatti et al., 2013)*. Pro- and anti-inflammatory cytokines were determined by Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, US), according to the manufacturer's instructions.

## 7.9. Statistical analysis

The results were expressed as mean ± standard deviation (SD), median and range, or geometric mean with 95% confidence interval (CI) as appropriate. Categorical variables were presented in terms of frequencies and percentages. All data were treated as paired samples from a crossover study. The two interventions were analyzed combining results obtained by the different periods in the two groups of subjects at different phases. The results were analyzed within each group using two-tailed student's T-test. Differences between baseline characteristics, according to randomization, and absolute variations (mean baseline values subtracted from mean values after intervention) were estimated by independent sample T-test. The Spearman (R) test was used for correlation between variations of vitamin B12 and interleukin-6 levels.

Histograms and box-plots were used to assess the distributional assumptions and to check for possible outliers. To compare the effect of the two different diets, a general linear model adjusted for order of treatment and weight change (for biochemical, oxidative and inflammatory parameters) was conducted. Since these tests assume normal data distribution, non-distributed data have been transformed into logs and further analyses were made with the processed data. However, to facilitate interpretation, the log data were again converted to the original scale (antilog) and presented as geometric means with 95% confidence intervals (CI). Losses were included in the intention-to-treat but not in the per-protocol analyses.

The possible dietary carryover effect, that is, the effect that considers whether the impact of the first treatment is still present when the patient enters the second treatment period, was analysed. We evaluated the sequence effect, which considers whether the impact of the vegetarian diet and the Mediterranean diet was different when the order of administration changed. This effect was estimated by comparing the geometric mean change difference between treatments in the vegetarian diet group and in the Mediterranean diet group, after adjustment for order of treatment.

Subgroup analyses were performed in order to analyze possible differences of changes according to some characteristics of the study population such as age (≤50 years; >50 years), sex (females; males), categories of BMI (25-29.9 kg/m$^2$; >30 kg/m$^2$), obesity status (class I: 30-34.9 kg/m$^2$; class II: 35-39.9 kg/m$^2$; class III: >40 kg/m$^2$), years of education (≤13 years; >13 years), physical activity (absent or light/moderate), civil status (married; not married), total cholesterol (≤190 mg/dl; >190 mg/dl), LDL-cholesterol (≤115 mg/dl; >115 mg/dl), triglycerides (≤150 mg/dl; >150 mg/dl), glucose (<110 mg/dl; 110-126 mg/dl). P-values <0.05 were considered statistically significant.

The statistical package PASW 20.0 for Macintosh (SPSS Inc., Chicago, IL) was used.

# 8. RESULTS

## 8.1. Participants

One hundred forty-three subjects were assessed for eligibility but 25 were excluded because they did not meet the inclusion criteria or because they refused to participate. Hence, 118 subjects were enrolled and entered the randomization. Figure 8.1 shows the enrolment of participants in the study.

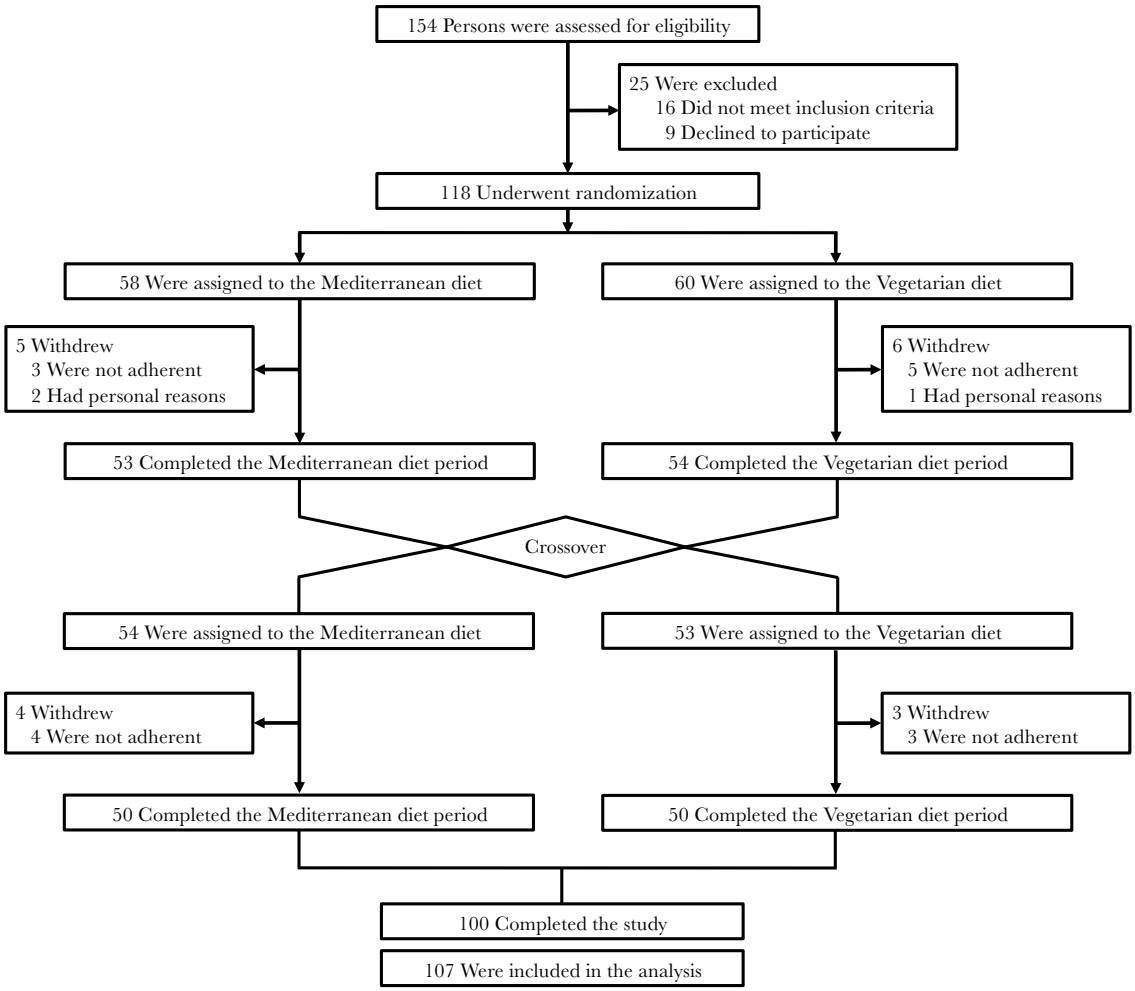


**Figure 8.1.** Flow-chart of study participants

During the first phase, 6 participants assigned to the vegetarian diet and 5 randomized to the Mediterranean diet retired because of personal reasons or because they were not adherent to the proposed diet. A total of 107 subjects completed the first phase. After crossover, 3 subjects in the vegetarian diet arm and 4 in the Mediterranean diet were excluded because of poor compliance to the diet. Finally, a total of 100 participants (50 subjects for each intervention) completed the entire study. The total participation rate at the end of the study was 84.7%.

Baseline characteristics of the studied population, according to the first dietary intervention, are shown in Table 8.2.

**Table 8.2.** Baseline characteristics of the study population according to randomization

| Characteristic | All (n=118) | VD (n=60) | MD (n=58) | p-value |
|---|---|---|---|---|
| **Age,** yr (median and range) | 50 (21-75) | 49.5 (24-70) | 52 (21-75) | 0.57 |
| **Female sex,** n (%) | 92 (78) | 49 (81.7) | 43 (74.1) | 0.37 |
| **Weight,** kg | 83.9 ± 16.8 | 82.9 ± 16.0 | 84.9 ± 17.7 | 0.63 |
| **BMI,** kg/m$^2$ | 30.6 ± 4.9 | 30.1 ± 4.7 | 31.1 ± 5.1 | 0.29 |
| Obese (≥ 30 kg/m$^2$), n (%) | 57 (48.3) | 27 (45) | 30 (51.7) | 0.58 |
| **Fat mass,** % | 37.9 ± 8.2 | 38.0 ± 8.4 | 37.9 ± 8.0 | 0.66 |
| **Dietary profile** | | | | |
| **Total energy**, kcal/day | 2071.3 ± 548.4 | 2101.9 ± 527.4 | 2039.5 ± 572.2 | 0.39 |
| **Carbohydrate**, % of energy | 47.2 ± 8.7 | 47.3 ± 8.6 | 47 ± 8.9 | 0.96 |
| **Protein**, % of energy | 17.1 ± 4.3 | 16.9 ± 4.7 | 17.2 ± 3.9 | 0.51 |
| **Total fat**, % of energy | 37 ± 6.2 | 36.8 ± 6.2 | 37.3 ± 7.1 | 0.75 |
| **Saturated fat**, % of energy | 8.1 ± 2.9 | 7.8 ± 2.2 | 8.4 ± 3.4 | 0.57 |
| **Total cholesterol**, mg/day | 202.7 ± 109.2 | 198.8 ± 94.5 | 206.8 ± 124.9 | 0.96 |
| **Risk factors** | | | | |
| **Current smokers,** n (%) | 17 (14.4) | 6 (19) | 11 (19) | 0.20 |
| **Absent or light physical activity,** n (%) | 107 (90.7) | 54 (90) | 53 (91.4) | 0.78 |
| **Total cholesterol >190 mg/dl,** n (%) | 90 (76.3) | 47 (78.3) | 43 (74.1) | 0.59 |
| **LDL cholesterol >115 mg/dl,** n (%) | 87 (73.7) | 45 (75) | 42 (72.4) | 0.75 |
| **Triglycerides >150 mg/dl,** n (%) | 34 (28.8) | 16 (26.7) | 18 (31) | 0.60 |
| **Glucose 110-126 mg/dl,** n (%) | 17 (14.4) | 6 (10) | 11 (19) | 0.17 |
| **Blood biomarkers** | | | | |
| **Total cholesterol,** mg/dl | 212.3 ± 38.3 | 210.5 ± 34.1 | 214.2 ± 42.4 | 0.59 |
| **LDL-cholesterol,** mg/dl | 131.7 ± 32.7 | 130.8 ± 30.2 | 132.7 ± 35.5 | 0.76 |
| **Triglycerides,** mg/dl | 125.0 ± 62.8 | 124.4 ± 64.9 | 125.6 ± 61.1 | 0.78 |

p-value calculated using the independent sample T-test
VD denotes vegetarian diet, MD Mediterranean diet, BMI Body Mass Index and LDL Low-Density Lipoprotein

Most participants were females (78%). The mean age was 50 years (range 21-75) and the BMI was on average 30.6 ± 4.9 kg/m$^2$, with 48.3% of the population that was obese. At the time of enrollment, the mean daily caloric intake of subjects was 2,071 ± 548 kcal, with a mean percentage intake of carbohydrates, protein, fats and saturated fats of 47.2%, 17.1%, 37% and 8.1%, respectively. Daily intake of cholesterol was on average 202.7 mg. The vast majority of the studied population was sedentary (90.7%), and nearly 15% were smokers. Regarding risk factors for cardiovascular disease, 76.3% had high levels of total cholesterol, 73.7% high levels of LDL cholesterol and 28.8% high levels of triglycerides.

There were no significant differences between participants firstly assigned to the vegetarian and Mediterranean diets in all the demographics, anthropometric parameters, biomarkers and cardiovascular disease risk factors, as well as in the composition of the diet consumed before the onset of the intervention.

## 8.2. Dietary intake

The analysis of dietary profiles at the end of both interventions demonstrated that participants significantly decreased total energy, total fat, saturated fat, and cholesterol intakes compared to baseline, as reported in Table 8.3.

However, no significant difference in the variations were observed between the groups, apart from cholesterol intake, which showed a most significant decrease in the vegetarian diet group, and protein intake which increased in the Mediterranean diet group and decreased in the vegetarian diet group.

**Table 8.3.** Variations in dietary intake according to first randomization

| | All (n=118) | VD (n=60) | MD (n=58) | p value° |
|---|---|---|---|---|
| **Total energy**, kcal/day | | | | |
| Change (after-before) | -542.3 ± 513.4 * | -586.3 ± 486.9 * | -496.7 ± 539.8 * | 0.18 |
| **Carbohydrate**, % of energy | | | | |
| Change (after-before) | 6.1 ± 8.7 | 6.8 ± 8.6 | 5.3 ± 8.9 | 0.37 |
| **Protein**, % of energy | | | | |
| Change (after-before) | 0.1 ± 4.6 | -1.5 ± 4.7 | 1.4 ± 3.9 | 0.001 |
| **Total fat**, % of energy | | | | |
| Change (after-before) | -7.3 ± 6.7 * | -6.3 ± 6.1 * | -8.2 ± 7.1 * | 0.15 |
| **Saturated fat**, % of energy | | | | |
| Change (after-before) | -0.7 ± 6.8 * | -0.31 ± 2.2 * | -1.0 ± 3.3 * | 0.41 |
| **Total cholesterol**, mg/day | | | | |
| Change (after-before) | -20.6 ± 69.7 * | -105.6 ± 91.5 * | -49.7 ± 121.8 * | 0.001 |

Data are reported as mean ± standard deviation
VD denotes vegetarian diet, MD Mediterranean diet
* denotes $p<0.05$ for change (after the first intervention period vs before the first intervention period)
° denotes difference between change in VD group and change in MD group

## 8.3. Body weight and body composition

Figure 8.2 shows the changes of the anthropometric parameters at the end of the study, after combining data from both intervention periods.

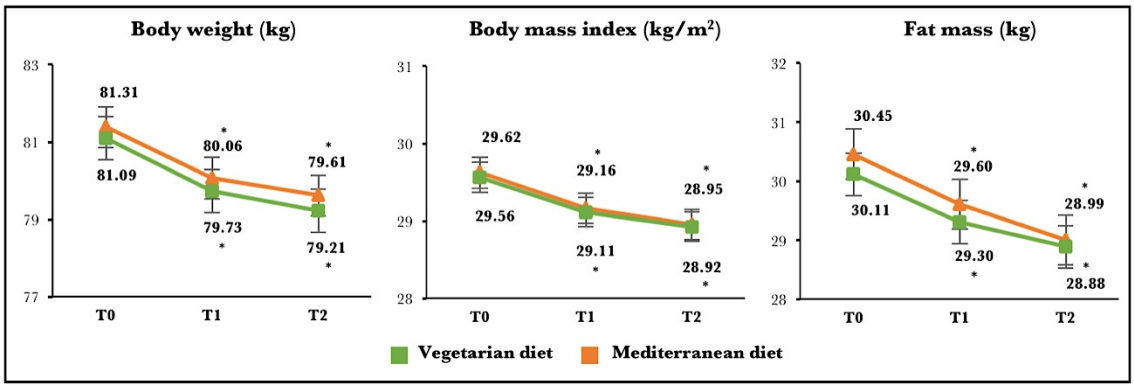


**Figure 8.2.** Body weight, BMI and fat mass changes according to diet group
(* denotes $p<0.05$ for change within each group)

No significant difference between the 2 diets was found, as both vegetarian and Mediterranean diets resulted similarly effective, with a difference between the vegetarian and

Mediterranean diets in end-of-diet values of 0.11 kg for weight (p=0.946), 0.03 kg/m$^2$ for BMI (p=0.841) and 0.23 kg for fat mass (p=0.501). As regards change within each group, a significant body weight reduction of -1.88 kg (95% CI -2.42; -1.35) and -1.77 kg (95% CI -2.29; -1.25) with a significant BMI reduction of -0.64 kg/m$^2$ (95% CI -0.84; -0.43) and -0.67 kg/m$^2$ (95%CI -0.86; -0.47), and a significant fat mass reduction of -1.23 kg (95% CI -1.67; -0.80) and -1.46 kg (95% CI -1.93; -1.01) for the vegetarian and the Mediterranean diets was reported.

Subgroup analyses showed no significant differences in changes of all the anthropometric parameters. No differences in physical activity were observed during the experimentation.

## 8.4. Lipid profile

Changes in the lipid profile during the two treatment periods are shown in Figure 8.3. After adjustment for order of treatment and weight change, the vegetarian diet reported a significant decrease (-2.57%) in total cholesterol levels [post: 202.55 (95% CI 195.98-209.56) mg/dl vs pre: 207.89 (95% CI 200.74-215.29) mg/dl; p=0.025] compared to the Mediterranean diet, which resulted in a non-significant change after 3 months of intervention [post: 205.30 (95% CI 198.34-212.72) mg/dl vs pre: 205.41 (95% CI 197.95-212.94) mg/dl; p=0.990].

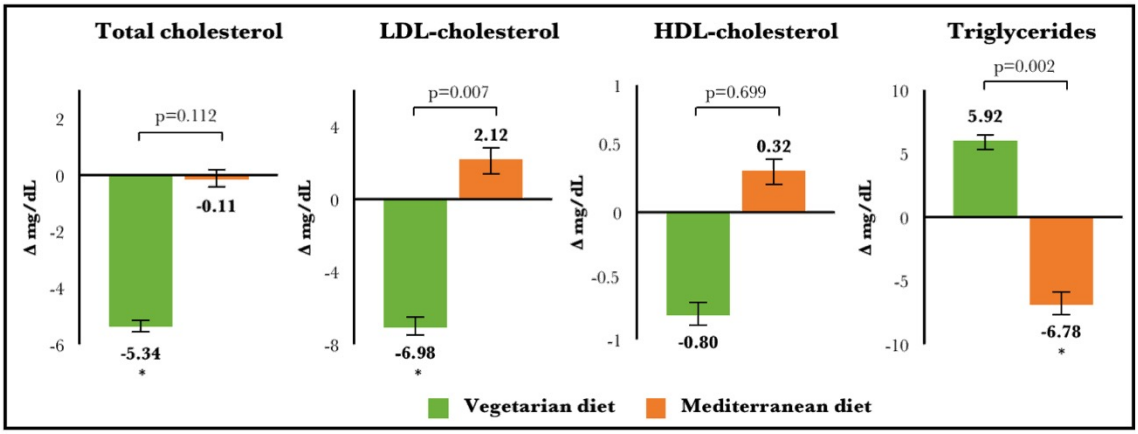


**Figure 8.3.** Lipid profile changes according to diet group
(* denotes p<0.05 for change within each group)

The same results were obtained for LDL-cholesterol levels, with the vegetarian diet which resulted in a significant decrease (-5.44%) [post: 121.27 (95% CI 114.89-127.87) mg/dl vs. pre: 128.25 (95% CI 114.89-134.83) mg/dl; p=0.003] while the Mediterranean diet reported only non-significant variations [post: 125.84 (95% CI 119.22-132.69) mg/dl compared to pre: 123.72 (95% CI 116.86-130.84) mg/dl; p=0.303]. In this case, the difference between the delta changes of the two intervention periods was statistically significant (p=0.011).

On the other hand, the Mediterranean diet resulted in a significant decrease (-5.91%) in triglyceride levels [post: 107.88 (95% CI 98.59-118.16) mg/dl vs. pre: 114.66 (95% CI 104.38-125.96) mg/dl); p=0.015], compared to the vegetarian diet that showed an increasing trend, despite not significant [post: 114.66 (95% CI 104.27-126.09) mg/dl vs pre: 108.74 (95% CI 99.29-119.10) mg/dl; p=0.079]. The difference between delta changes in triglyceride levels was statistically significant (p=0.006).

There was no significant change in HDL-cholesterol for either diets even though there was an increasing trend for the Mediterranean diet [post: 53.41 (95% CI 51.21-55.70) mg/dl vs. pre: 53.09 (95% CI 50.65-55.70) mg/dl; p=0.645] compared to the vegetarian diet which showed a non-significant decreasing trend [post: 52.56 (95% CI 50.30-54.93) mg/dl vs pre: 53.36 (95% CI 51.26-55.48) mg/dl; p=0.263]. The ratio of total cholesterol to HDL-cholesterol showed non-significant variations during either the vegetarian diet [post: 3.99 (95% CI 3.80-4.17) vs. pre: 4.02 (95% CI 3.84-4.21); p=0.56] or the Mediterranean diet [post: 3.96 (95% CI 3.79-4.13) vs. pre: 4.00 (3.81-4.19); p=0.49], despite a decreasing trend was evidenced in both periods of intervention.

Subgroup analyses showed that lipid profile changes during the vegetarian diet were more evident in men, in participants older than 50 years, in non-smokers, in sedentary subjects, and in participants with a BMI above 30 $kg/m^2$, with most significant results in class I obese subjects.

## 8.5. Biochemical profile

Changes of all the other biochemical parameters after the vegetarian diet period, including hematologic variables, vitamins, iron status, minerals, liver function tests, uric acid, and glycemic profile, are reported in Table 8.4.

**Table 8.4.** Changes in biochemical parameters after the vegetarian diet period

| | **VD pre** (n=104) | **VD post** (n=104) | **p-value** |
|---|---|---|---|
| **WBC**, x10$^3$/mm$^3$ | 6.06 (5.80-6.34) | 6.22 (5.96-6.48) | 0.131 |
| **RBC**, x10$^6$/mm$^3$ | 4.70 (4.63-4.77) | 4.67 (4.60-4.74) | 0.095 |
| **Hemoglobin**, g/dl | 13.65 (13.42-13.90) | 13.57 (13.31-13.83) | 0.157 |
| **Hematocrit**, % | 41.14 (40.49-41.76) | 40.94 (40.25-41.60) | 0.198 |
| **Folate**, ng/ml | 6.67 (6.04-7.36) | 7.08 (6.44-7.78) | 0.057 |
| **Vitamin B12**, pg/ml | 380.7 (357.2-404.2) | 361.4 (340.4-383.4) | 0.011 |
| **Ferritin**, ng/ml | 50.30 (41.14-61.56)) | 51.01 (41.70-62.43) | 0.761 |
| **Iron**, µg/dl | 79.44 (74.22-84.94) | 78.10 (72.53-84.18) | 0.632 |
| **Sodium**, mEq/l | 139.1 (138.8-139.4) | 139.5 (139.2-139.9) | 0.008 |
| **Potassium**, mEq/l | 4.25 (4.20-4.30) | 4.26 (4.21-4.31) | 0.610 |
| **Calcium**, mg/dl | 8.83 (8.73-8.93) | 8.84 (8.76-8.91) | 0.994 |
| **Magnesium**, mg/dl | 2.02 (1.99-2.05) | 2.05 (2.02-2.08) | 0.054 |
| **AST**, U/l | 17.46 (16.35-18.65) | 17.17 (15.91-18.52) | 0.543 |
| **ALT**, U/l | 27.47 (25.64-29.43) | 27.83 (26.00-29.78) | 0.580 |
| **Gamma-GT**, U/l | 23.13 (20.76-25.76) | 24.61 (22.13-27.39) | 0.087 |
| **Uric acid**, mg/dl | 4.15 (3.96-4.35) | 4.03 (3.85-4.22) | 0.038 |
| **Insulin,** µU/ml | 9.38 (8.59-10.25) | 8.89 (8.10-9.76) | 0.126 |
| **Glucose,** mg/dl | 89.93 (87.71-92.11) | 90.47 (88.15-92.94) | 0.477 |
| **HOMA-Index** | 2.08 (1.89-2.29) | 1.99 (1.79-2.21) | 0.225 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using a general linear model adjusted for order of treatment and weight change
VD denotes vegetarian diet, WBC White Blood Cells, RBC Red Blood Cells, AST Aspartate aminotransferases, ALT Alanine aminotransferases, GT Glutamyl Transferase, and HOMA-IR Homeostasis Model Assessment of Insulin Resistance

A non-significant decreasing trend in the erythrocyte count, hemoglobin and hematocrit levels, as well as in glucose profile during the intervention period with the vegetarian diet, was evidenced. As far as vitamin B12 is concerned, there was a significant decrease by 5.06%. For minerals, significant changes were observed only for sodium, which increased by 0.42 mEq/l

(+0.3%). Interestingly, during the vegetarian diet period, participants had a significant reduction in uric acid (-0.12 mg/dl; -2.89%). No significant changes were reported for all the other parameters investigated.

With regard to the Mediterranean diet period, as depicted in Table 8.5 there was a significant increase in magnesium levels (+1.95%). No significant changes were reported for all the other parameters investigated.

**Table 8.5.** Changes in biochemical parameters after the Mediterranean diet period

| | **MD pre** (n=103) | **MD post** (n=103) | **p-value** |
|---|---|---|---|
| **WBC**, x$10^3$/$mm^3$ | 6.34 (6.07-6.61) | 6.25 (5.98-6.53) | 0.383 |
| **RBC**, x$10^6$/$mm^3$ | 4.70 (4.62-4.77) | 4.72 (4.64-4.80) | 0.175 |
| **Hemoglobin**, g/dl | 13.68 (13.46-13.90) | 13.71 (13.49-13.92) | 0.683 |
| **Hematocrit**, % | 41.06 (40.41-41.72) | 41.26 (40.61-41.97) | 0.151 |
| **Folate**, ng/ml | 6.81 (6.17-7.51) | 7.26 (6.60-7.99) | 0.075 |
| **Vitamin B12**, pg/ml | 376.9 (356.0-399.0) | 389.9 (367.6-413.6) | 0.087 |
| **Ferritin**, ng/ml | 56.71 (46.25-69.55) | 53.84 (43.90-66.09) | 0.204 |
| **Iron**, µg/dl | 79.68 (73.48-86.40) | 78.57 (73.63-83.76) | 0.696 |
| **Sodium**, mEq/l | 139.2 (138.8–139.5) | 139.3 (138.9–139.5) | 0.881 |
| **Potassium**, mEq/l | 4.25 (4.20-4.30) | 4.28 (4.22-4.33) | 0.322 |
| **Calcium**, mg/dl | 8.86 (8.84-8.94) | 8.84 (8.75-8.93) | 0.584 |
| **Magnesium**, mg/dl | 2.01 (1.98-2.04) | 2.05 (2.02-2.09) | 0.002 |
| **AST**, U/l | 17.18 (15.94-18.50) | 17.73 (16.76-18.77) | 0.220 |
| **ALT**, U/l | 27.52 (25.51-29.73) | 27.55 (25.87-29.37) | 0.967 |
| **Gamma-GT**, U/l | 24.39 (21.89-27.19) | 24.51 (21.96-27.36) | 0.871 |
| **Uric acid**, mg/dl | 4.10 (3.92-4.31) | 4.20 (4.00-4.41) | 0.126 |
| **Insulin,** µU/ml | 9.75 (8.98-10.58) | 9.29 (8.51-10.16) | 0.180 |
| **Glucose,** mg/dl | 90.56 (88.23-92.94) | 90.83 (88.68-93.13) | 0.642 |
| **HOMA-Index** | 2.18 (1.99-2.39) | 2.09 (1.78-2.30) | 0.225 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using a general linear model adjusted for order of treatment and weight change

Differences between vegetarian and Mediterranean diets are reported in Table 8.6. Statistically significant differences between delta changes in the two treatment periods were observed for erythrocyte count (p=0.038), hematocrit (p=0.036), vitamin B12 (p=0.001), and uric acid (p=0.001).

**Table 8.6.** Differences between vegetarian and Mediterranean diets after 3 months of intervention

| | **VD $\Delta_{post-pre}$** | **MD $\Delta_{post-pre}$** | **ΔVD - ΔMD** | **p ($\Delta_{VD}$ vs. $\Delta_{MD}$)** |
|---|---|---|---|---|
| **WBC**, x10³/mm³ | 0.16 (-0.09; 0.36) | -0.09 (-0.29; 0.14) | 0.25 | 0.420 |
| **RBC**, x10⁶/mm³ | -0.03 (-0.07; 0.01) | 0.02 (-0.01; 0.07) | -0.05 | 0.038 |
| **Hemoglobin**, g/dl | -0.08 (-0.20; 0.04) | 0.03 (-0.08; 0.13) | -0.11 | 0.194 |
| **Hematocrit**, % | -0.20 (-0.53; 0.13) | 0.20 (-0.08; 0.57) | -0.40 | 0.036 |
| **Folate**, ng/ml | 0.41 (-0.33; 0.95) | 0.45 (-0.13; 1.03) | -0.04 | 0.857 |
| **Vitamin B12**, pg/ml | -19.29 (-35.64; -5.43) | 13.03 (-3.22; 29.25) | -32.32 | 0.001 |
| **Ferritin**, ng/ml | 0.71 (-3.97; 7.89) | -2.87 (-11.91; 0.99) | 3.58 | 0.480 |
| **Iron**, µg/dl | -1.34 (-5.98; 4.77) | -1.11 (-8.64; 2.39) | -0.23 | 0.620 |
| **Sodium**, mEq/l | 0.42 (0.12; 0.80) | 0.10 (-0.34; 0.40) | 0.32 | 0.074 |
| **Potassium**, mEq/l | 0.01 (-0.05; 0.08) | 0.03 (-0.02; 0.08) | -0.02 | 0.957 |
| **Calcium**, mg/dl | 0.01 (-0.09; 0.08) | -0.02 (-0.09; 0.05) | 0.03 | 0.650 |
| **Magnesium**, mg/dl | 0.03 (-0.01; 0.06) | 0.04 (0.02; 0.08) | -0.01 | 0.484 |
| **AST**, U/l | -0.29 (-0.99; 0.94) | 0.55 (-0.86; 1.00) | -0.84 | 0.505 |
| **ALT**, U/l | 0.36 (-1.29; 2.10) | 0.03 (-2.26; 0.77) | 0.33 | 0.445 |
| **Gamma-GT**, U/l | 1.48 (-0.55; 4.19) | 0.12 (-1.98; 2.02) | 1.36 | 0.649 |
| **Uric acid**, mg/dl | -0.12 (-0.24; -0.03) | 0.10 (-0.02; 0.23) | -0.22 | 0.001 |
| **Insulin,** µU/ml | -0.49 (-1.22; 0.27) | -0.46 (-1.12; 0.41) | -0.03 | 0.423 |
| **Glucose,** mg/dl | 0.54 (-0.97; 2.43) | 0.27 (-1.13; 1.53) | 0.27 | 0.599 |
| **HOMA-Index** | -0.09 (-0.27; 0.13) | -0.09 (-0.26; 0.13) | 0 | 0.374 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using the independent sample T-test

Subgroup analyses showed that the variation in vitamin B12 levels after the vegetarian diet phase was more apparent among overweight subjects (especially among class I obese subjects), men, and participants aged less than 50 years.

## 8.6. Oxidative stress profile

Changes in oxidative stress profile after the vegetarian diet period are reported in Table 8.7. After 3 months of intervention, there was a significant reduction in TBARS (-0.39 pg/ml; -22.54%) and leukocyte-derived ROS (-59.62 RFU; -8.42%) levels.

**Table 8.7.** Changes in oxidative stress parameters after the vegetarian diet period

| | **VD pre** (n=104) | **VD post** (n=104) | **p-value** |
|---|---|---|---|
| **TBARs**, pg/ml | 1.73 (1.48-2.01) | 1.34 (1.13-1.59) | <0.0001 |
| **TAC**, µmol/ml | 14.40 (13.76-15.04) | 14.06 (13.36-14.79) | 0.158 |
| **L-derived ROS**, RFU | 707.69 (659.84-759.00) | 648.07 (598.24-702.05) | 0.033 |
| **M-derived ROS**, RFU | 1247.63 (1169.11-1332.75) | 1187.97 (1104.35-1279.21) | 0.176 |
| **G-derived ROS**, RFU | 1844.57 (1718.14-1980.29) | 1737.15 (1611.62-1870.57) | 0.117 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using general linear model adjusted for order of treatment and weight change
VD denotes vegetarian diet, TBARs ThioBarbituric Acid Reactive Substances, TAC Total Antioxidant Capacity, L Leukocytes, ROS Reactive Oxygen Species, M Monocytes, G Granulocytes and RFU Relative Fluorescence Units

On the other hand, the Mediterranean diet period resulted in a significant reduction of only TBARS levels (-0.32 pg/ml; -19.05%) (Table 8.8). As well as for the vegetarian diet, TAC, M-derived and G-derived ROS levels showed decreasing but not significant trends.

**Table 8.8.** Changes in oxidative stress parameters after the Mediterranean diet period

| | **MD pre** (n=104) | **MD post** (n=104) | **p-value** |
|---|---|---|---|
| **TBARs**, pg/ml | 1.68 (1.44-1.95) | 1.36 (1.15-1.60) | 0.003 |
| **TAC**, µmol/ml | 14.35 (13.71-15.03) | 14.24 (13.61-14.89) | 0.548 |
| **L-derived ROS**, RFU | 684.03 (635.24-736.57) | 666.47 (622.66-714.08) | 0.505 |
| **M-derived ROS**, RFU | 1230.28 (1149.41-1315.54) | 1171.45 (1099.93-1248.88) | 0.129 |
| **G-derived ROS**, RFU | 1775.79 (1654.08-1904.55) | 1674.05 (1571.84-1782.91) | 0.086 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using general linear model adjusted for order of treatment and weight change

Comparing delta changes in the two treatment periods, no difference between vegetarian and Mediterranean diets were observed (Table 8.9)

**Table 8.9.** Differences between vegetarian and Mediterranean diets after 3 months of intervention

| | **VD** $\Delta_{\text{post-pre}}$ | **MD** $\Delta_{\text{post-pre}}$ | **ΔVD - ΔMD** | **p** ($\Delta_{\text{VD}}$ vs. $\Delta_{\text{MD}}$) |
|---|---|---|---|---|
| **TBARs**, pg/ml | -0.39 (-0.65; -0.09) | -0.32 (-0.58; -0.06) | -0.07 | 0.882 |
| **TAC**, µmol/ml | -0.34 (-0.67; 0.20) | -0.11 (-0.49; 0.22) | -0.23 | 0.599 |
| **L-derived ROS**, RFU | -59.6 (-105.2; 6.7) | -17.6 (-79.9; 26.3) | -42.06 | 0.669 |
| **M-derived ROS**, RFU | -59.7 (-137.13; 49.44) | -58.8 (-150.4; 9.51) | -0.83 | 0.622 |
| **G-derived ROS**, RFU | -107.4 (-236.4; 30.9) | -101.7 (-247.4; -5.7) | -5.68 | 0.637 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using the independent sample T-test

## 8.7. Inflammatory profile

Changes in the inflammatory profile after the vegetarian diet period are reported in Table 8.10. A significant decreasing trend in MCP-1 (-9.9%), VEGF (-11.4%), IP-10 (-9.4%) and IFN-gamma (-25.7%) levels was reported. On the contrary, a statistically significant increase by 71.4 % was observed for IL-4 levels, as well as an increase by 37.6% for IL-17. There were no significant changes for all the other inflammatory parameters.

**Table 8.10.** Changes in inflammatory parameters after the vegetarian diet period

| | **VD pre** (n=104) | **VD post** (n=104) | **p-value** |
|---|---|---|---|
| **IL-1ra,** pg/ml | 11.62 (9.82-13.76) | 10.33 (8.76-12.18) | 0.132 |
| **IL-4,** pg/ml | 0.07 (0.05-0.09) | 0.12 (0.09-0.16) | <0.0001 |
| **IL-6,** pg/ml | 0.74 (0.60-0.92) | 0.81 (0.66-1.00) | 0.497 |
| **IL-8,** pg/ml | 3.39 (2.72-4.22) | 2.86 (2.27-3.61) | 0.207 |
| **IL-10,** pg/ml | 1.71 (1.32-2.21) | 1.83 (1.41-2.39) | 0.565 |
| **IL-12,** pg/ml | 15.46 (13.40-17.85) | 15.43 (13.40-17.74) | 0.967 |
| **IL-17,** pg/ml | 3.70 (2.82-4.86) | 5.09 (4.14-6.26) | 0.027 |
| **MCP-1,** pg/ml | 21.24 (18.90-23.88) | 19.13 (17.03-21.50) | 0.042 |
| **MIP-1beta,** pg/ml | 48.91 (43.90-54.43) | 45.11 (41.26-49.25) | 0.071 |
| **VEGF,** pg/ml | 39.88 (33.72-47.18) | 35.30 (29.99-41.55) | 0.008 |
| **TNF-alpha,** pg/ml | 3.05 (2.23-4.17) | 3.50 (2.92-4.18) | 0.160 |
| **IP-10,** pg/ml | 479.62 (435.72-527.95) | 434.41 (393.07-480.10) | 0.029 |
| **IFN-gamma,** pg/ml | 3.58 (2.87-4.46) | 2.66 (2.06-3.43) | 0.041 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using a general linear model adjusted for order of treatment and weight change
VD denotes vegetarian diet, MD Mediterranean diet, IL Interleukin, MCP Monocyte Chemoattractant Protein, MIP Macrophage Inflammatory Protein, VEGF Vascular Endothelial Growth Factor, TNF Tumor Necrosis Factor, IP Interferon-Gamma Induced Protein, and IFN Interferon

With respect to the Mediterranean diet, decreases in IL1-ra (-20.4%), IL-12 (-12.9%), IL-17 (-36.3%), MCP-1 (-21%), VEGF (-15.67%), and MIP-1beta (-13.2%) levels were reported. As well as for the vegetarian diet, IL-4 levels significantly increased by 37.6% (Table 8.11).

**Table 8.11.** Changes in inflammatory parameters after the Mediterranean diet period

| | **MD pre** (n=103) | **MD post** (n=103) | **p-value** |
|---|---|---|---|
| **IL-1ra,** pg/ml | 13.45 (11.43-15.82) | 10.70 (9.23-12.39) | 0.009 |
| **IL-4,** pg/ml | 0.07 (0.05-0.09) | 0.12 (0.09-0.16) | <0.0001 |
| **IL-6,** pg/ml | 0.84 (0.68-1.04) | 0.75 (0.63-0.90) | 0.303 |
| **IL-8,** pg/ml | 3.35 (2.69-4.18) | 3.01 (2.42-3.75) | 0.346 |
| **IL-10,** pg/ml | 1.81 (1.37-2.37) | 1.50 (1.14-1.95) | 0.178 |
| **IL-12,** pg/ml | 16.48 (14.11-19.26) | 14.35 (12.45-16.59) | 0.025 |
| **IL-17,** pg/ml | 5.51 (4.54-6.69) | 3.51 (2.68-4.61) | 0.005 |
| **MCP-1,** pg/ml | 22.76 (20.05-25.87) | 17.98 (16.17-19.97) | <0.0001 |
| **MIP-1beta,** pg/ml | 52.40 (47.66-57.57) | 45.47 (41.06-50.40) | 0.001 |
| **VEGF,** pg/ml | 42.86 (35.80-51.32) | 36.16 (30.51-42.91) | 0.002 |
| **TNF-alpha,** pg/ml | 3.20 (2.53-4.04) | 2.86 (2.12-3.87) | 0.456 |
| **IP-10,** pg/ml | 475.33 (427.95-527.95) | 447.20 (407.48-490.78) | 0.153 |
| **IFN-gamma,** pg/ml | 2.53 (1.93-3.30) | 3.22 (2.58-4.00) | 0.087 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using a general linear model adjusted for order of treatment and weight change
MD denotes Mediterranean diet, MCP Monocyte Chemoattractant Protein, MIP Macrophage Inflammatory Protein, VEGF Vascular Endothelial Growth Factor, TNF Tumor Necrosis Factor, IP Interferon-Gamma Induced Protein, and IFN Interferon

Table 8.12 shows differences between delta changes in the two treatment periods. A significant difference between diets was observed for IL-17 levels, which had an opposite tendency during the two phases of intervention, as evidenced by a significant increase during the vegetarian diet period and a significant decrease during the Mediterranean diet period. No statistically significant differences between the two dietary interventions for all the other inflammatory parameters were reported.

**Table 8.12.** Differences between vegetarian and Mediterranean diets after 3 months of intervention

| | **VD $\Delta_{post-pre}$** | **MD $\Delta_{post-pre}$** | **ΔVD - ΔMD** | **p ($\Delta_{VD}$ vs. $\Delta_{MD}$)** |
|---|---|---|---|---|
| **IL-1ra,** pg/ml | -1.29 (-4.54; 0.60) | -2.75 (-7.78; -1.87) | 1.46 | 0.369 |
| **IL-4,** pg/ml | 0.05 (0.01; 0.12) | 0.05 (0.02; 0.11) | 0 | 0.989 |
| **IL-6,** pg/ml | 0.07 (-0.22; 0.19) | -0.09 (-0.44; -0.09) | 0.16 | 0.058 |
| **IL-8,** pg/ml | -0.53 (-2.42; 1.84) | -0.34 (-2.41; 0.82) | 0.19 | 0.710 |
| **IL-10,** pg/ml | 0.12 (-0.69; 1.23) | -0.31 (-2.32; -0.36) | 0.43 | 0.067 |
| **IL-12,** pg/ml | -0.03 (-2.67; 1.30) | -2.13 (-5.72; -0.65) | 2.10 | 0.134 |
| **IL-17,** pg/ml | 1.39 (-0.62; 1.84) | -2.00 (-3.51; -0.01) | 3.39 | 0.007 |
| **MCP-1,** pg/ml | -2.11 (-5.62; 0.44) | -4.78 (-10.78; -4.11) | 2.67 | 0.197 |
| **MIP-1beta,** pg/ml | -3.80 (-11.14; -1.42) | -6.93 (-11.90; -2.77) | 3.13 | 0.486 |
| **VEGF,** pg/ml | -4.58 (-11.65; -2.70) | -6.70 (-17.79; -5.84) | 2.12 | 0.634 |
| **TNF-alpha,** pg/ml | 0.45 (-3.20; 0.53) | -0.34 (-1.05; 2.48) | 0.79 | 0.248 |
| **IP-10,** pg/ml | -45.21 (-111.5; 14.37) | -28.13 (-115.0; 4.20) | 17.08 | 0.397 |
| **IFN-gamma,** pg/ml | -0.92 (-3.52; 0.14) | 0.69 (-0.79; 1.51 | 1.61 | 0.107 |

Data are reported as geometric mean and 95% confidence interval (CI)
p-value calculated using the independent sample T-test
VD denotes vegetarian diet, MD Mediterranean diet, IL Interleukin, MCP Monocyte Chemoattractant Protein, MIP Macrophage Inflammatory Protein, VEGF Vascular Endothelial Growth Factor, TNF Tumor Necrosis Factor, IP Interferon-Gamma Induced Protein, and IFN Interferon

Overall, the vegetarian diet resulted in a reduction of 8 out of 13 cytokines with 6 that reached the statistical significance, whereas the Mediterranean diet determined a reduction of 11 out of 13 pro- and anti-inflammatory cytokines, with 7 that reached the statistical significance. Variations of pro- and anti-inflammatory cytokines that reported a significant change are shown in Figure 8.4. Between-group comparisons were calculated using the independent sample T-test.

No carryover effects were detected for all the anthropometric, biochemical, inflammatory and oxidative stress parameters investigated.

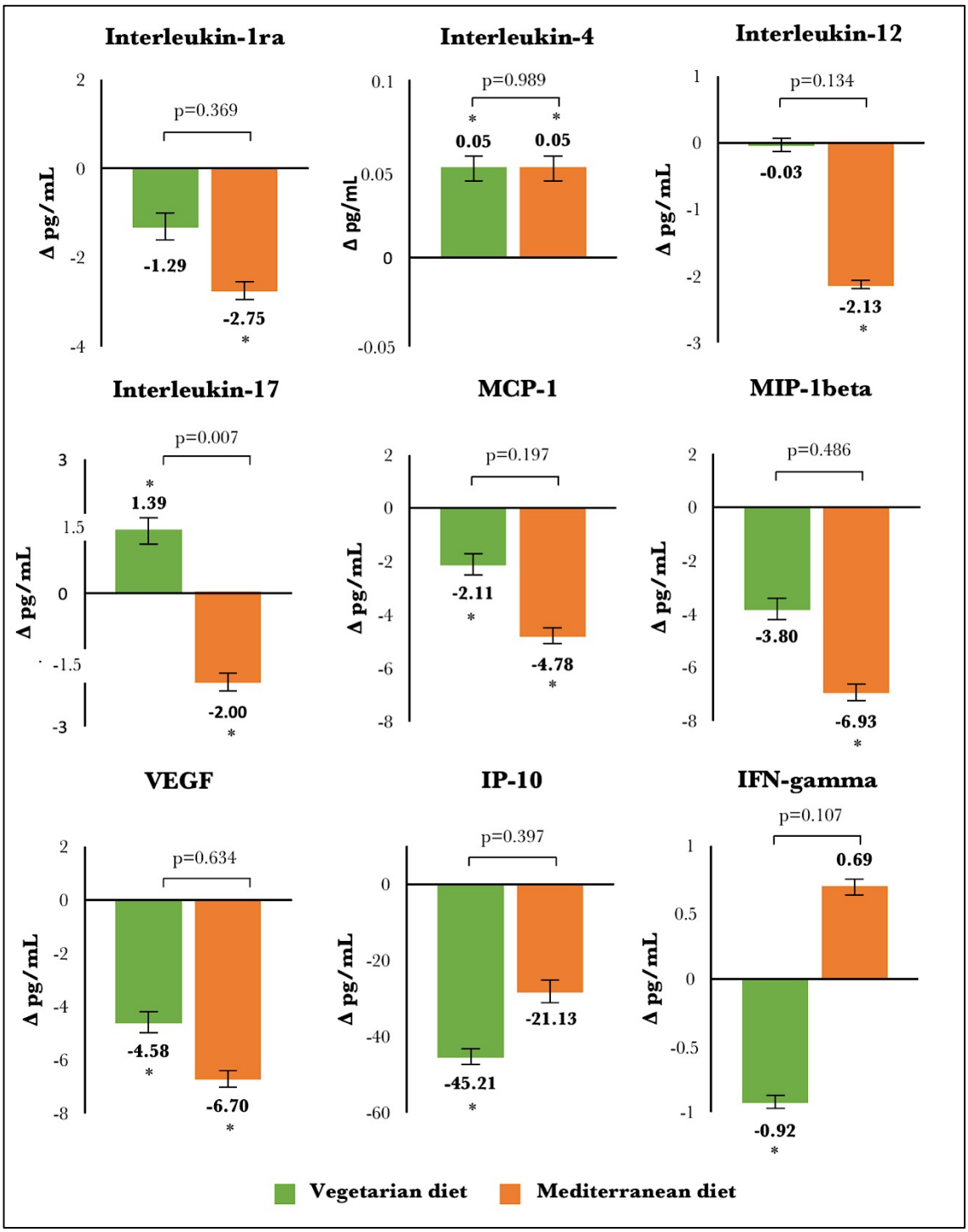


**Figure 8.4.** Variations of pro- and anti-inflammatory cytokines that reported a significant change after 3 months of dietary intervention, according to the diet group (* denotes $p<0.05$ for change within each group)

## 8.8. Cardiovascular risk profile

Both diets resulted in a significant improvement of the cardiovascular risk profile of the study participants. Forty-six participants, during the vegetarian diet (44.2% of the subjects who completed the vegetarian diet period) and 35, during the Mediterranean diet (34% of the participants in whom the Mediterranean diet was initiated) modified their risk category by reaching the target values recommended by the European Society of Cardiology (*Piepoli et al., 2016*) for at least 1 cardiovascular risk factor (total cholesterol ≤190 md/dl, LDL-cholesterol ≤115 mg/dl, triglyceride levels ≤150 mg/dl, glucose levels ≤110 mg/dl, BMI <25 kg/m$^2$).

Of these, during the vegetarian diet period, 16 reached the target values for total cholesterol, 17 for LDL-cholesterol, 6 for triglyceride levels, and 14 for BMI. As for Mediterranean diet, only 7 subjects reached the target values for total cholesterol, 6 for LDL-cholesterol, 8 for triglyceride levels, and 10 for BMI.

## 8.9. Compliance

During the study, 18 (15.3%) participants reported a less-than-optimal compliance to the prescribed diets and were excluded at different time points from the study. The comparison of the baseline characteristics between participants who completed the study and those who were excluded for not being adherent showed significant differences in age, BMI and physical activity (Table 8.13).

Participants who did not finish the study were significantly younger (41 vs. 52 years), had a higher BMI (33.1 vs 30.1 kg/m2) and had significantly more sedentary lifestyles than the participants who completed the study. By conducting all the analyses after the inclusion of the non-adherent participants, through an intention-to-treat analysis, the results of both the anthropometric and circulating biomarkers did not substantially change

**Table 8.13.** Baseline characteristics of the study population according to the completion of the study

| Characteristic | All (n=118) | Completers (n=100) | Non-completers (n=18) | p value |
|---|---|---|---|---|
| **Age,** yr (median and range) | 50 (21-75) | 52 (21-75) | 41 (28-57) | 0.004 |
| **Female sex,** n (%) | 92 (78) | 76 (76) | 16 (88.9) | 0.35 |
| **Weight,** kg (mean ± SD) | 83.9 ± 16.8 | 82.7 ± 16.1 | 90.6 ± 19.2 | 0.12 |
| **BMI,** kg/m$^2$ (mean ± SD) | 30.6 ± 4.9 | 30.1 ± 4.6 | 33.1 ± 6.0 | 0.027 |
| Obese (≥ 30 kg/m$^2$), n (%) | 57 (48.3) | 46 (46) | 11 (61.1) | 0.31 |
| **Fat mass,** % (mean ± SD) | 37.9 ± 8.2 | 37.3 ± 8.4 | 41.5 ± 5.5 | 0.07 |
| **Dietary profile** | | | | |
| **Total energy**, kcal/day (mean ± SD) | 2071.3 ± 548.4 | 2071.4 ± 567.8 | 2070.3 ± 438.8 | 0.58 |
| **Carbohydrate**, % of energy (mean ± SD) | 47.2 ± 8.7 | 47.7 ± 8.8 | 44.3 ± 7.7 | 0.07 |
| **Protein**, % of energy (mean ± SD) | 17.1 ± 4.3 | 16.9 ± 4.3 | 17.8 ± 4.4 | 0.37 |
| **Total fat**, % of energy (mean ± SD) | 37 ± 6.2 | 36.7 ± 6.5 | 39.1 ± 7.0 | 0.18 |
| **Saturated fat**, % of energy (mean ± SD) | 8.1 ± 2.9 | 8.2 ± 2.9 | 7.9 ± 2.9 | 0.78 |
| **Total cholesterol**, mg/day (mean ± SD) | 202.7 ± 109.2 | 199.9 ± 110.9 | 218.4 ± 101.3 | 0.45 |
| **Risk factors** | | | | |
| **Current smokers,** n (%) | 17 (14.4) | 14 (14) | 3 (16.7) | 0.50 |
| **Absent or light physical activity,** n (%) | 107 (90.7) | 89 (89) | 15 (100) | 0.45 |
| **Total cholesterol >190 mg/dl,** n (%) | 90 (76.3) | 76 (76) | 14 (77.8) | 0.10 |
| **LDL-cholesterol >115 mg/dl,** n (%) | 87 (73.7) | 75 (75) | 11 (66.7) | 0.56 |
| **Triglycerides >150 mg/dl,** n (%) | 34 (28.8) | 28 (28) | 6 (33.3) | 0.78 |
| **Glucose 110-126 mg/dl,** n (%) | 17 (14.4) | 13 (13) | 1 (5.6) | 0.69 |
| **Blood biomarkers** | | | | |
| **Total cholesterol,** mg/dl (mean ± SD) | 212.3 ± 38.3 | 214.1 ± 37.5 | 202.6 ± 42.2 | 0.43 |
| **LDL-cholesterol,** mg/dl (mean ± SD) | 131.7 ± 32.7 | 133.2 ± 33.3 | 123.5 ± 29 | 0.37 |
| **Triglycerides,** mg/dl (mean ± SD) | 125.0 ± 62.8 | 123.8 ± 60.7 | 131.6 ± 75.4 | 0.92 |

p-value calculated using the independent sample T-test

# 9. DISCUSSION AND CONCLUSION

The vegetarian and Mediterranean dietary profiles have a common background since they are both aimed at maximizing the consumption of whole, plant-based foods and discouraging consumption of meat and meat products as well as refined and processed foods. Health benefits and, in particular, the potential to significantly reduce the risk of cardiovascular disease have been established for both diets. However, the efficacy of nutritional interventions based on vegetarian and Mediterranean diets had not yet been tested on the same group of subjects. This PhD project compared for the first time the effectiveness of a vegetarian diet versus a similar Mediterranean diet in improving the cardiovascular risk profile of a clinically healthy omnivorous population.

The main findings of the trial were that 3-months' intervention with a vegetarian and a Mediterranean diet showed similar reduction in total body weight, BMI, and total fat mass, with no differences between diets. In addition, while the vegetarian diet seemed to be more effective in reducing total and LDL-cholesterol levels, only the Mediterranean diet determined a significant reduction in triglyceride levels. Regarding oxidative stress and inflammatory profiles, both diets contributed to a significant improvement in most parameters, with the vegetarian diet which caused a slightly greater reduction in oxidative stress markers and the Mediterranean diet which reported a reduction in a larger number of pro- and anti-inflammatory cytokines. A significant difference between diets was, however, reported only for IL-17.

Our findings regarding the beneficial effect of the vegetarian and the Mediterranean diets on body weight, BMI, and fat mass are in line with the scientific literature. Indeed, a recent meta-analysis by Barnard et al. identified 6 trials that analyzed a vegetarian-like period and reported a significant reduction in total body weight with an average mean reduction of 3.4 kg *(Barnard et al., 2015)*. In the same year, the results of an additional meta-analysis including 12 randomized controlled trials which involved subjects who followed vegetarian diets reported similar findings, with a mean reduction of 2.2 kg with respect to the non-vegetarian group

*(Huang et al., 2016)*. Despite a similar significant trend, we found a slightly lesser reduction of body weight among subjects following the vegetarian diet (-1.74 kg). This difference in terms of body weight change can be explained by several factors: first, by the different duration of interventions; second, by the lack of a comparable diet for most studies; and third, by the fact that the results of previous intervention studies investigated not only the vegetarian diet but also the vegan diet and other form of vegetarianism. As regards the Mediterranean diet, many intervention studies demonstrated the beneficial effects of this dietary pattern on body weight and several anthropometric measurements *(Dinu et al., 2017)*. Our results confirm these findings, and extend the evidence of a beneficial effect of Mediterranean diet on body weight also in comparison with an isocaloric vegetarian diet.

As for lipid profile, we found a beneficial effect for both diets, with the vegetarian diet determining a significant reduction of total cholesterol and LDL-cholesterol, and the Mediterranean diet of triglyceride levels. Accordingly, a previous meta-analysis that included 11 randomized trials conducted on subjects who followed vegetarian diets versus control diets reported a significant effect on lowering total cholesterol, LDL-cholesterol, and HDL-cholesterol, but not triglyceride levels *(Wang al., 2015)*. Pharmacological studies showed that a reduction of 1 mmol/l (38.67 mg/dl) in total cholesterol and LDL-cholesterol levels decreases from 30% to 27% the risk of all cardiovascular disease *(Schwingshackl et al., 2013)*. On the other hand, the observed null effect of the vegetarian diet on triglyceride levels may be explained by the paradoxical effects reported by other studies of an increased level of circulating triglyceride levels due to the high content of carbohydrate and total fat that occurs when meat and meat products are eliminated from the diet *(Parks et al., 2001)*. However, in this trial the two diets were not essentially different in terms of weekly portions consumed by these food groups, so the null effect on triglyceride seems not to follow such hypothesis. On the contrary, we observed a beneficial effect of the Mediterranean diet on triglyceride levels as otherwise reported by previous intervention studies *(Dinu et al., 2017)*.

As expected, the intervention period with the vegetarian diet determined in the study population a significant reduction in vitamin B12 levels. Such reduction, despite clinically irrelevant and within the normal range values, confirms that the vegetarian diet may determine lower levels of this vitamin, as previously reported by other studies *(Pawlak et al., 2014)*. This issue warrants further investigations, since over an extended period a decrease in vitamin B12 associated with the vegetarian diet can determine a deficiency which may be clinically relevant. Indeed, the official position of scientific societies and agencies is unequivocal: subjects following a vegetarian diet should be screened for vitamin B12 deficiency and eventually encouraged to use fortified foodstuffs or supplements to ensure adequate vitamin B12 intake (*Rizzo et al., 2016)*.

With respect to the oxidative profile, the vegetarian diet seemed to result in a slightly greater reduction of oxidative stress markers with respect to the Mediterranean diet, with a significant reduction in TBARs and ROS derived from leukocytes. According to our knowledge, to date there are no other trials that evaluated these parameters after a period of intervention with a vegetarian diet in healthy subjects, while several results have already been obtained for the Mediterranean diet *(Estruch et al., 2010)*. On the other hand, a calorie-restricted vegetarian diet had greater capacity to improve oxidative stress parameters compared with a conventional diabetic diet over 24 weeks in a group of 74 patients with type 2 diabetes *(Kahleova et al., 2011*). As regards observational studies, Kim et al. *(2012)* investigated the long-term effect of eating a vegetarian diet on oxidative stress, finding lower levels of diacron reactive oxygen metabolites (d-ROM) in vegetarians than in omnivores.

Finally, this is the first study that evaluated a large pattern of pro- and anti-inflammatory cytokines in a dietary intervention study that includes a vegetarian diet. Both diets imposed an improvement of the inflammatory profile but the Mediterranean diet seemed to determine a reduction in a larger number of pro- and anti-inflammatory cytokines with respect to the vegetarian diet. The possible explanation of the lesser reduction observed during the vegetarian diet can be related to the reduced circulating levels of vitamin B12 detected during the vegetarian diet intervention. Several studies in the past reported a strict association between

vitamin B12 and inflammation, possibly through the modulation of the metabolic cycle of homocysteine *(Lee et al., 2016)*. On the other hand, a relationship between higher levels of IL-6 and lower levels of vitamin B12 has been previously reported *(Gori et al., 2005)* and supported by our results, since we observed an inverse and significant correlation between variations of IL-6 and vitamin B12 levels ($R=0.22$; $p=0.026$). Thus, it can be postulated that the vegetarian diet, determining a decrease in vitamin B12 levels increase homocysteine levels, with a consequent worsening of the inflammatory profile.

The strengths of this project include the crossover design, the comparability between the two diets in terms of total energy and macronutrients, the high rate of adherence, and the various parameters analyzed in the same group of subjects at different time points. In addition, it included the largest cohort of omnivorous subjects who underwent a period with a vegetarian diet so far. On the other hand, we are aware that 3 months of intervention is a limited period and permits only to suggest possible interpretation of the results. Further studies with a larger population and a longer duration are thus needed to confirm these results.

In conclusion, the identification of the best heart-healthy diet is still a challenging and highly relevant preventive health issue. The results of my PhD project show that both vegetarian and Mediterranean diets are effective in reducing body weight, BMI, and fat mass, with no significant differences between them. However, the vegetarian diet resulted more effective in reducing LDL-cholesterol levels, whereas the Mediterranean diet determined a significant reduction in triglyceride levels. The global challenges of diet-related diseases, especially cardiovascular disease, present enormous health and economic burdens and emphasize the imperative of prioritizing nutrition in clinical care, advocacy, research, and policy. Clinical behavior-change efforts, health system changes, novel technologies, and robust policy strategies must complement and facilitate individual food choices toward healthy dietary patterns such as Mediterranean and vegetarian diets, which together will reduce cardiometabolic disease and economic burdens across the population.

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# LIST OF PUBLICATIONS

1. Sofi F, Dinu M, Pagliai G, Cesari F, Gori AM, Sereni A, Becatti M, Fiorillo C, Marcucci R, Casini A. Low-calorie Vegetarian vs. Mediterranean diets for reducing body weight and improving cardiovascular risk profile: the CARDIVEG study. Circulation *(in press)*

2. Dinu M, Pagliai G, Macchi C, Sofi F. Commuting physical activity and multiple health outcomes: a meta-analysis. Br J Sports Med *(in press)*

3. Longo UG, Sofi F, Dinu M, Berton A, Cipriani G, Salvatore G, Denaro V. 2012 Alpine junior world ski championship: nutritional habits and performance in elite skiers. *(submitted)*

4. Dinu M, Whittaker A, Pagliai G, Giangrandi I, Colombini B, Gori AM, Fiorillo C, Becatti M, Casini A, Benedettelli S, Sofi F. Khorasan wheat products' consumption and non-alcoholic fatty liver disease (NAFLD): a randomized, double-blind trial. *(submitted)*

5. Longo UG, Sofi F, Dinu M, Candela V, Salvatore G, Cimmino M, Denaro V. Functional performance, anthropometric parameters and contribution to team success among elite goalkeepers of the Italian "SERIE A", season 2016-2017. *(submitted)*

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