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Unravelling the impact of two dietary intervention programs on salt intake

Shámila Faruk Ismael

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Avaliação do impacto de dois programas de intervenção nutricional no consumo de sal

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Shámila Faruk Ismael

Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto

Orientadora: Professora Doutora Cláudia Sofia Marques

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Personal contribution in the project presented in this dissertation

The candidate declares that had a decisive contribution in the accomplishment of the experimental work (designing and execution of the clinical study), as well as in the interpretation and discussion of the results presented in this dissertation. The candidate also actively contributed to the writing of this work.

Likewise, the candidate participated actively in the project "*Cerveja sem álcool no controle da Diabetes: Estudo clínico aleatorizado*" initially proposed as the dissertation theme. The change of theme is due to the fact that the study is not yet completed and there is still a new phase of recruitment of participants in progress.

Index

Figure index i
Table index ii
Resumo iii
Abstract v
Abbreviations vi
1. Introduction 1
1.1. Background 1
1.2. Hypertension 4
1.2.1. Dietary habits 6
1.2.2. Obesity 8
1.2.3. Physical Inactivity 10
1.2.4. Smoking 11
1.2.5. Diabetes Mellitus 11
1.2.6. Alcohol 13
1.2.7. Cholesterol 13
1.3. "Menos Sal Portugal" Project 14
2. Aims 15
3. Methods 16
3.1. Study design 16
3.2. Participants 16
3.3. Study protocol 17
3.4. Intervention 17
3.4.1. Salt-reduction Program 17
3.4.2. Healthy Lifestyle Program 18
3.5. Urinary sodium excretion 19
3.6. Blood Pressure measurements 19
3.7. Anthropometric measurements 19
3.7.1. Height 20
3.7.2. Body weight 20
3.7.3. Waist circumference 20
3.8. Questionnaires 20

3.9. Outcomes 21
3.10. Statistical analysis 21
4. Results 23
5. Discussion 30
6. Conclusion 35
References ..... - 1 -
Funding ..... - 13 -
Acknowledgements ..... - 13 -

Figure index

Figure 1 – Formation and progression of atherosclerotic plaque……3

Figure 2 – Association between obesity and CVD……10

Figure 3 – Mechanisms of CVD development from diabetes……12

Figure 4 – Study design……22

Figure 5 – Estimated salt intake post-intervention……24

Figure 6 – Adherence to Mediterranean diet……25

Figure 7 – Bodyweight loss according to dyslipidaemia……26

Table index

Table 1 – Inclusion and exclusion criteria……16

Table 2 – Baseline characteristics……23

Table 3 – Secondary outcomes……27

Table 4 – Food groups consumption evaluated by PREDIMED questionnaire……28

Table 5 – Bodyweight loss and variation in outcomes……29

Resumo

Introdução: As doenças cardiovasculares são a principal causa de mortalidade no mundo. Dentre os fatores de risco que contribuem para a fisiopatologia desse grupo de doenças, a hipertensão é o fator com maior impacto. A hipertensão e consequentemente as doenças cardiovasculares podem ser prevenidas no âmbito de um estilo de vida saudável. A população que reside nos países desenvolvidos, como Portugal, alterou os seus hábitos alimentares aumentando a ingestão de alimentos processados ricos em sal que podem afetar diretamente a pressão arterial. Neste contexto, este estudo tem como objetivos a avaliação do impacto de dois diferentes programas de educação nutricional no consumo estimado de sal, no peso, no perímetro abdominal e na adesão ao padrão alimentar mediterrânico.

Métodos: Realizou-se um estudo experimental, paralelo, com uma subamostra do estudo "Menos Sal Portugal" (ClinicalTrials.gov as NCT03830021) que envolveu 121 adultos residentes na região metropolitana de Lisboa com idades entre 20 e 70 anos, que foram alocados aleatoriamente em um dos dois programas de intervenção nutricional (Programa de Redução de Sal e Programa de Estilo de Vida Saudável). O consumo de sal foi estimado através da excreção urinária de sódio por 24 horas. A colheita de urina, o questionário PREDIMED e a medição do peso corporal, perímetro abdominal e pressão arterial foram analisadas antes da intervenção e após a intervenção (12 semanas).

Resultados: A ingestão de sal reduziu significativamente no Programa de Estilo de Vida Saudável. O peso corporal e o índice de massa corporal diminuíram significativamente após as duas intervenções e houve um aumento do PREDIMED *score*. Os indivíduos que reduziram mais peso tiveram uma maior redução no perímetro abdominal e na pressão arterial sistólica e diastólica.

Conclusão: Os programas de intervenção nutricional promovem hábitos alimentares mais saudáveis e têm um impacto positivo na redução dos fatores de risco que contribuem para as doenças cardiovasculares.

Palavras-chave: Doenças cardiovasculares, hipertensão, programas de intervenção nutricional, nutrição comunitária, consumo de sal, perda de peso

Abstract

Background: Cardiovascular diseases are the main cause of mortality worldwide. Out of the risk factors that contribute to the physiopathology of this group of diseases, hypertension has the most impact. Hypertension and consequently cardiovascular diseases can be prevented by healthy lifestyle. Population living in developed countries, such as Portugal, have altered dietary habits increasing the intake of processed foods rich in salt, which can affect directly blood pressure. With this, the aims of this study were to evaluate the impact of two different nutritional educational programs in the estimated salt consumption, bodyweight, waist circumference and in the adherence to Mediterranean diet.

Methods: This study is parallel-controlled trial with a subsample of the project "*Menos Sal Portugal*" (ClinicalTrials.gov as NCT03830021) which involved 121 adults living in Lisbon Metropolitan area aged 20-70 years old that were randomly allocated in one of the two educational programs (Salt-reduction Program and Healthy Lifestyle Program). Salt intake was estimated by 24-h urinary sodium excretion. Urine collection, PREDIMED questionnaire and bodyweight, waist circumference and blood pressure measurements were analysed at baseline and after the intervention (12-weeks).

Results: Salt intake reduced significantly in the Healthy lifestyle Program. Bodyweight and body mass index were significantly decreased after both interventions whereas PREDIMED score increased. Subjects that reduced more weight had a higher decrease on waist circumference, systolic and diastolic blood pressure.

Conclusion: Dietary educational programs promote healthier dietary habits and have a positive impact in the reduction of risk factors that contribute to cardiovascular diseases.

Keywords: Cardiovascular diseases, hypertension, dietary educational programs, community-based nutrition, salt intake, bodyweight loss

Abbreviations

AHA – American Heart Association

CVD – Cardiovascular diseases

DALY – Disability-adjusted life years

T2DM –Type 2 Diabetes Mellitus

ICAM – Intercellular adhesion molecule-1

IL-6 – Interleukin-6

LDL-c – Low-density lipoprotein cholesterol

NF-κB – Nuclear factor kappa-light-chain-enhancer of activated B cells

PHYSA – Portuguese Hypertension and Salt Study

RAAS – Renin-angiotensin-aldosterone system

ROS – Reactive oxygen species

SNS – Sympathetic nervous system

TNF-α – Tumour necrosis factor alpha

WHO – World Health Organization

1. Introduction

1.1. Background

Non-communicable diseases are the leading cause of mortality worldwide accounting for 40.5 million of the 56.9 million deaths in 2016[1]. Of the non-communicable diseases, cardiovascular diseases (CVD) are those with most impact on the mortality rate[1].

A report from European Society of Cardiology showed that 45% of all deaths were caused by CVD, with a higher prevalence in women (49%) than in men (40%)[2]. In Portugal, CVD is the main cause of mortality, with stroke and coronary heart disease accounting for 6.1% and 6.0%, respectively, of total disability-adjusted life years (DALY)[3].

CVD is a group of heterogeneous diseases where there is an abnormal function of the circulatory system with severe repercussions in health and life expectancy. This group of diseases can be divided into three major groups, depending on the affected arteries:

Coronary heart disease or coronary artery disease characterized by poor or no blood flow on coronary artery to the heart tissue. It can be caused by a plaque, e.g. - as a result of atherosclerosis, leading to acute myocardial infarction that evolves into necrosis of myocardial tissue. Suddenly block of coronary artery, described as heart attack, also evolves into myocardial infraction. This disease is associated with angina that happens when there's a temporary loss of blood supply to the heart causing chest pain[4].

Cerebrovascular disease which involves interruption of the blood supply to part of the brain and may result in a stroke or transient ischemic attack. The block of the artery most commonly arises from process of thromboembolism, where a formed blood clot becomes dislocated and blocks a cerebral artery. Narrowing of the intracerebral artery with atherosclerotic plaque may increase the risk and lead to local formation of a blood clot. There may be irreversible damage to the brain tissue of the affected area[4].

Peripheral vascular disease which is a result of atherosclerotic plaque formation that causes obstruction of large arteries that supplies blood to other regions apart from the myocardium and the brain. This can cause pain during exercise and in more severe situations, may require amputation of the dead tissues[4].

Regardless of the affected artery, CVD share a common pathophysiology involving atherosclerosis and thrombosis (or clotting)[5].

Atherosclerosis is a chronic inflammatory disease characterized by the narrowing of medium and large-sized arteries due to plaque formation. It has been proposed that atherosclerosis begins with endothelial dysfunction which can be triggered by the increase of low-density lipoprotein cholesterol (LDL-c) in circulation[6–8]. With the high concentration of LDL-c, these lipoproteins start to deposit in tunica intima and become oxidized by the reactive oxygen species (ROS) and metalloproteases, which activates endothelial cells to express receptors for blood leukocytes on their surface and thus, allowing monocytes and T-helper cells to move into tunica intima layer. These monocytes become macrophages that have scavenger receptors which engulf oxidized LDL molecules and become foam cells that release ROS and inflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin-1, hence, increasing inflammation[6–8]. Foam cells also release growth factors such as insulin growth factor-1 that lead to proliferation and migration of smooth muscle cells into the tunica intima and eventually ruptures, releasing lipids, inflammatory cytokines and growth factors, evolving into a plaque. During this process, this plaque can grow and produce ischemia by insufficient blood flow from high oxygen demand or by rupturing and eventually resulting in thrombosis where coagulation happens to stop the rupture, therefore creating a thrombus (clot) that obstruct blood flow. The endothelium dysfunction results also in production of less nitric oxide (NO), an essential vasodilator, which causes constriction of blood vessels[6–8] (**Figure 1**).

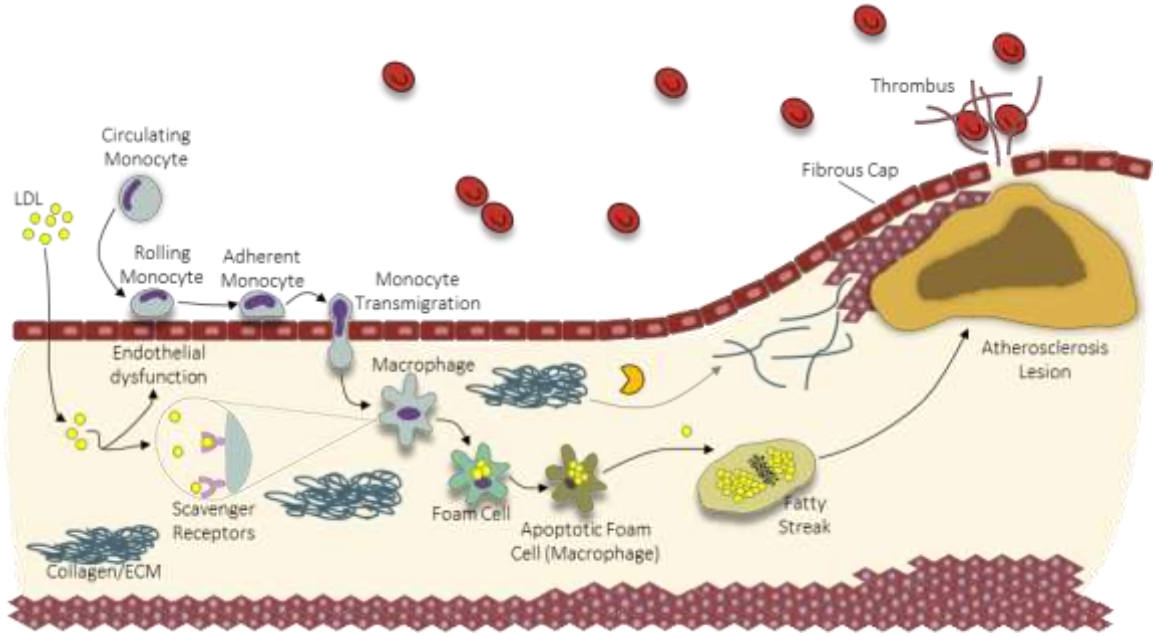

Figure 1 - Formation and progression of atherosclerotic plaque. LDL = low-density lipoprotein; BM = bone marrow; CVD = cardiovascular diseases; VCAM-1 = vascular cell adhesion molecule-1; ICAM-1 = intercellular adhesion molecule-1; ECM = extracellular matrix; VSMC = vascular smooth muscle cells. Adapted from Head *et al.*[9]

There has been substantial improvement in CVD outcomes in recent years, however, it remains the leading cause for morbidity and mortality globally. Hence, identifying those with higher risk and moving individuals toward ideal cardiovascular health is essential for prevention of these diseases[10]. Prevention strategies occur at population level but must also be directed to the individual throughout its life to slow the development of CVD by promoting a healthy lifestyle[10].

Changes in the lifestyle of the population that lives in developed countries results in the decrease of CVD rate. This observation has led to extensive research on prevention, diagnosing risk factors and predictors of CVD which can help detect risk patients in time and prevent the disease effectively[5]. A report from World Health Organization (WHO) estimates that it is possible to prevent over 75% of premature CVD and that reducing risk factors can help ameliorate growing CVD burden on both general population and healthcare providers[11].

When it comes to identifying high risk individuals, the European Society of Cardiology recommends the use of SCORE (Systematic Coronary Risk Evaluation) system that estimates the 10-year risk of fatal CVD for apparently healthy individuals[12]. This consists

in a table that contributes to evaluate the risk of an individual having CVD in a 10-year interval by accessing risk factors associated with this group of diseases. The risk factors involve gender, age, systolic blood pressure, total cholesterol and smoking status[12,13].

1.2. Hypertension

Hypertension is considered the modifiable risk factor with most impact in CVD risk[14].

According to results from the first Portuguese Health Examination Survey 2015, the prevalence of this important risk factor in Portuguese population between 25 and 74 years old is 36%[15].

Studies have shown that even systolic blood pressure as low as 115 mm Hg and 75 mm Hg as diastolic blood pressure can increase the risk of mortality from stoke or ischemic heart disease. Recently meta-analysis has also shown that even prehypertension is enough to increase cardiovascular risk[16,17].

Thus, American College of Cardiology and American Heart Association (AHA) released new guidelines for diagnostic and management of hypertension in order to identify individuals in risk as early as possible. It is considered normal blood pressure when systolic blood pressure is under 120 mm Hg and diastolic under 80 mm Hg, whereas when systolic raise to values between 120- and 129-mm Hg is considered elevated blood pressure and hypertension is considered when systolic blood pressure is over 130 mm Hg and diastolic over 80 mm Hg[18].

High blood pressure is a product of cardiac output and peripheral vascular resistance. The pathophysiology of this disease involves the impairment of renal pressure natriuresis, which is a feedback system where the high blood pressure induces an increase in sodium and water excretion by the kidney, leading to a reduction of the blood pressure. Pressure natriuresis can result from impaired renal function, inappropriate activation of hormones that regulate salt and water excretion by the kidney [such as those in the renin-angiotensin-aldosterone system (RAAS)], or excessive activation of the sympathetic nervous system (SNS)[19–21].

The baroreceptors present in kidney detect a decrease in arterial blood pressure and produce renin, that enters circulation and convert angiotensinogen produced by the liver into angiotensin I that circulates and contacts with the membrane bound enzyme angiotensin converting enzyme which converts angiotensin I into angiotensin II. Angiotensin II binds into angiotensin II receptors present in different cells and results in an increase of blood pressure by increasing sympathetic nervous system activity, vasoconstriction and aldosterone[21].

Hypertension contributes to cardiovascular risk trough atherosclerosis by different levels: to the development of endothelial dysfunction, fatty streaks, early atherosclerotic plaque, plaque progression and plaque rupture[22].

With hypertension, there are changes in hemodynamic forces which are perceived by the mechanoreceptors of endothelial cells. The activation of these receptors triggers intracellular pathways, especially the mitogen-activated protein kinase, that lead to phosphorylation of transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which modulates the expression of mechanosensitive genes[22]. These genes include ROS production from nicotinamide adenine dinucleotide phosphate-oxidase and other enzymes like xanthine oxidase, and a decrease in activity of the detoxifying enzyme superoxide dismutase. Excess availability of superoxide anions decreases NO bioavailability. NF-κB also increase expression of adhesion molecules, chemokines and inflammatory cytokines leading to atherosclerosis[22].

Therefore, it is important to identify individuals in risk of hypertension in order to prevent the development of the disease and reduce the risk of mortality due to CVD[23].

Because hypertension has an essential part in the progression of CVD, to reduce the bearing of this diseases work should begin by sensibilization of the individuals when it comes to modifiable risk factors to CVD, which are common to hypertension. The individual risk factors with most impact will be discussed next[24].

1.2.1. Dietary habits

Poor diet is one of the major risk factors for CVD and hypertension, accounting for 72 % of deaths attribute to this disease, in part as results of its effects on other risk factors that will be further mentioned[25].

According to the first Portuguese Health Examination Survey, dietary habits of Portuguese population are diverted from a heathy diet[26]. It is estimated that in Portugal bad food habits contribute to 15.8% of DALY, especially the excessive salt consumption[27,28]. Moreover, only 1 of every 2 people consume the 5 portions of fruit and vegetables recommended by WHO[26]. Furthermore, recent results from *Programa Nacional para a Promoção da Alimentação Saudável* estimate that the average Portuguese has drank 60 litres of soft drink and 4 kg of sugar per year[29].

Initially, it was taken a more reductionist approach whereby nutrition research focused on isolated nutrients instead of considering foods and the total diet. But because the impact of nutrients to cardiovascular health is related to the food matrix in which it was consumed, present dietary guidelines emphasize dietary pattern and food-based recommendations, although many still include recommendations for saturated fat, sodium, added sugar, and dietary cholesterol because these nutrients are over-consumed and have an negative impact in non-communicable diseases[25].

Studies have shown that dietary patterns such as Mediterranean diet, Dietary Approaches to Stop Hypertension and Alternative Healthy Eating Index are associated with reduced risk of CVD[30].

Because of the geographic location of Portugal, Mediterranean food pattern has been a part of its history[31]. However, according to a validated score to evaluate adherence to this food pattern, the adherence was low in all Portuguese regions[32].

Mediterranean food pattern is characterized by a high consumption of whole grains, fruits and vegetables, nuts, legumes and fish, with olive oil as the main type of fat, a moderate consumption of milk and dairy products, a low-to-moderate consumption of wine and low consumption of meat and meat products[33].

Studies have shown that fruits and vegetables[34], nuts[35], legumes, whole grains[36], olive oil[37] and wine are associated with a lower risk of CVD[38].

The abundance of plant-based foods and the moderate presence of animal products results in a higher ratio of unsaturated fat to saturated fat reducing CVD risk by lowering LDL-c levels, oxidative stress and inflammation from the limited content of heme groups which are related to oxidative reactions, damage proteins and cell membrane lipids[38]. The anti-oxidant and anti-inflammatory effect is especially due to the polyphenols content present in nuts, wine, fruits, vegetables and olive oil[39].

Mediterranean diet is also rich in vitamin C and E, and minerals such as potassium and magnesium, which is also associated with reduction of ROS and inflammation. These micronutrients are associated with lower blood pressure, insulin sensitivity and improvement in endothelial function[38].

Legumes, nuts, fruits and vegetables are good sources of fibre which means that these groups of foods improve cardiovascular health trough reduction in LDL-c, control of body weight and long-term weight maintenance by lowering energy intake and triggering satiety hormones[36,38].

Lastly, Mediterranean diet has also a positive impact on gut microbiota by the increase of short chain fatty acids that can increase fatty acid oxidation, decrease in the liver the *novo* fatty acid synthesis, plasma glucose and cholesterol levels. Additionally, this diet results in lower levels of urinary trimethylamine-N-oxide, suggested as biomarker of CVD since it may initiate atherosclerosis[38,40].

1.2.1.1. Salt intake

Although salt intake is not considered an independent risk factor for CVD, it is important to consider since studies have shown that it may directly increase risk of stroke from the effect in blood pressure[5]. Dietary salt intake is considered the major cause of raised blood pressure[41].

Results from the first Portuguese Health Examination Survey estimate that the average daily intake of salt is 7.3 g/d, with bread and toasts, cold cuts and soups as the foods that most contribute for this high consumption[26]. However, results from the Portuguese Hypertension and Salt study (PHYSA), where salt intake was calculated by the 24-h urinary sodium excretion, showed that the average salt consumption by Portuguese

population is 10.7 g/d, with a higher consumption in hypertensive individuals than in normotensive[42]. This represents more than twice of the recommended intake by WHO (5 g of salt per day)[43].

A reduction of the current high level of salt intake in the population is expected not only to have a short-term blood pressure lowering effect but also to slow the increase in blood pressure associated with ageing[41].

However, salt reduction in normotensive individuals may not be recommended as it causes only a small reduction and it might be harmful to health[41].

In salt sensitivity individuals, excess salt intake results in an expansion of extracellular fluid volume and increase cardiac output. Plasma fluid volume increases osmolality which creates more pressure leading to arterial stiffness resulting in damage of organs such as brain, kidney and heart. Since kidney is the major filtering organ, because of the constant pressure to excrete water there is an activation of RAAS, there is also an activation in SNS which together leads to higher blood pressure[44,45].

Additionally, excess salt intake results in oxidative stress and consequently in endothelial disfunction, follow-on a decrease of NO and more vasoconstriction[46].

In order to reduce the risk of CVD it is important to prevent hypertension progress by salt reduction, especially in hypertensive individuals. For this, it is necessary to promote healthy eating habits and reduction in consumption of foods with high salt content. Most of these foods are processed and ready-to-eat meals, canned products and smoked meat and fish. Substitution of salt for herbs and spices, and an increase in potassium rich foods results in less consumption of salt and reduces the impact of overconsumption, respectively[47].

1.2.2. Obesity

The prevalence of obesity has increased, reaching epidemic proportions worldwide[48]. In Portugal, the results from the first Portuguese Health Examination Survey conducted on adults aged 25-74 years showed that in 2015 the prevalence of overweight was 34.8 %

and obesity 22.3 %, while overweight was higher among men, women had higher obesity rates[26].

Obesity is typically defined according to body mass index (BMI), a normal BMI is between 18.5 and 24.9 kg/m^2, overweight a BMI between 25 and 29.9 kg/m^2, and obesity, a BMI higher or equal to 30 kg/m^2. As for the severity of obesity, if BMI is between 30 and 34.9 kg/m^2 is defined as grade I obesity, BMI between 35 and 39.9 kg/m^2 grade II obesity and BMI higher or equal to 40 kg/m^2 as grade III obesity. Additionally subcategories have been introduced due to rapid extend of this disease, if BMI is higher or equal to 50 kg/m^2 is considered grade IV and higher or equal to 60 kg/m^2, grade V obesity[49]. For people aged ≥ 65 years old it was proposed a different classification: BMI under 23 kg/m^2 is considered underweight, 24-30 kg/m^2 healthy weight and above 30 kg/m^2 overweight[50].

Because BMI does not account for adipose tissue distribution, there are alternatives measures of adiposity including waist-to-hip ratio and waist circumference. Central obesity is defined as waist circumference higher than 102 cm in men and 88 cm in women, or waist-to-hip ratio higher than 1.0 in men and 0.85 in women[51].

The risk of CVD increases with higher obesity grade, grade I obesity has 75% higher risk comparing with BMI under 25 kg/m^2 , this risk increases 2.5-fold with grade II and 4-fold with grade III[51].

Obesity is associated with the risk of CVD and hypertension due to an increase in the load of atherosclerotic plaques that is identified by greater macrophage infiltration and plaque instability[52]. Chronic low-grade inflammation appears to be what triggers, at least in part, to the increase in CVD risk by its contribution to endothelial dysfunction trough the effect of adipokines, mainly adiponectin and TNF-α which are secreted by fat tissue after macrophage recruitment[48,52]. Visceral fat accumulation is associated with insulin resistance induced by cytokines [interleukin-6 (IL-6), TNF-α and adiponectin), non-esterified fatty acid and retinol binding protein 4 that induce oxidative stress and subsequent endothelial dysfunction [plasminogen activator inhibitor-1 and intercellular adhesion molecule-1 (ICAM-1)]. The visceral fat accumulation, insulin resistance, liver-induced inflammation and alteration in lipid profile features may all lead to the premature atherosclerotic process, thus an increase in cardiovascular risk[48] (**Figure 2**).

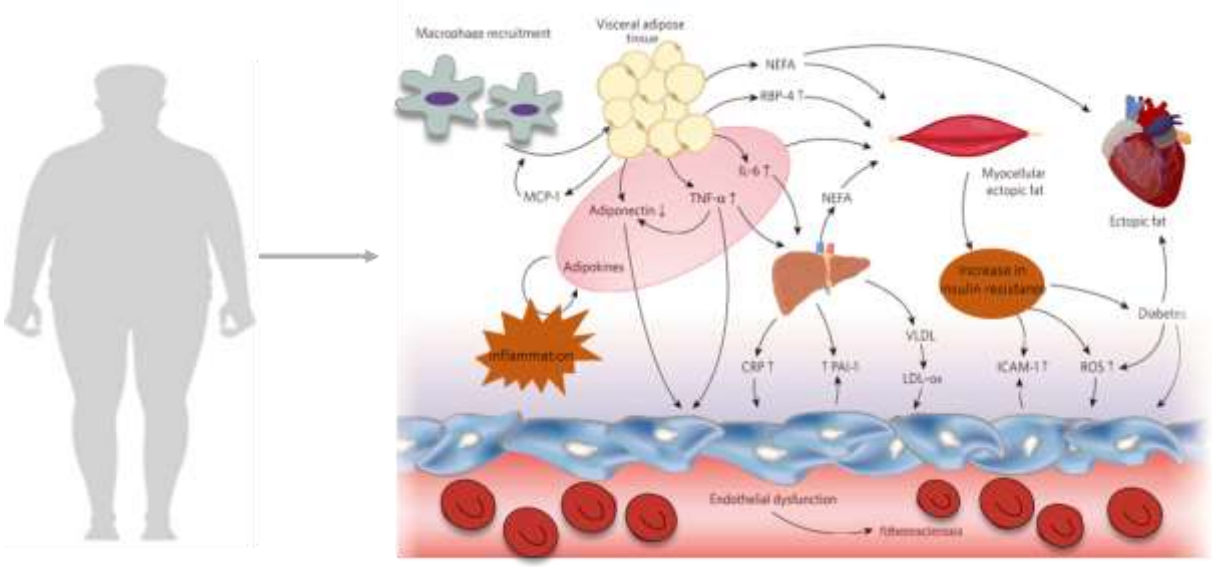

Figure 2 - Association between obesity and CVD. MCP-1 = monocyte chemoattractant protein-1; NEFA = non-esterified fatty acid; RBP-4 = retinol binding protein 4; TNF-α = tumour necrosis factor alpha; IL-6 = interleukin-6; VLDL = very low-density lipoprotein; LDL-ox = Oxidized low-density lipoprotein; CRP = C-reactive protein; PAI-1 = plasminogen activator inhibitor-1; ICAM-1 = intercellular adhesion molecule-1; ROS = reactive oxygen species. Adapted from Gaal *et al.*[48]

1.2.3. Physical Inactivity

Lack of moderate-to vigorous physical activity has been identified as an important risk factors in the development of cardiovascular disease, up to an increase of 63%[53,54]. In Portugal, a recent report indicates that only 27% of the population older than 14 years old are considered active (1-hour or more of moderate activity daily/ 30 minutes of vigorous activity daily), 30% are considered moderate active (30 minutes of moderate activity almost every day) and almost half of the population (43%) are sedentary or inactive[26].

Exercise reduce the release of inflammatory cytokines [TNF-α, IL-6, Interferon-γ], chemokines (Interleukin-8), adhesion molecules [vascular cell adhesion molecule-1 and ICAM-1] and angiogenic factors (vascular endothelial growth factor and endothelial progenitor cells), which together contribute to atherosclerosis and plaque formation, thus improving cardiovascular health[55,56].

Exercise also increases lipid particles size and endothelial NO synthesis that reduces vasoconstriction, which is promoted by the increase of insulin sensitivity[57].

Hence, it is essential that health professionals promote physical activity adapted to the individual in order to reduce the risk of CVD (primary prevention) and CVD events (secondary prevention)[58].

1.2.4. Smoking

Smoking it is well recognised as a risk factor for CVD, even 1 cigarette a day increase the risk of myocardial infraction, this is also valid for second-hand smokers. Growing evidence shows that electronic cigarettes also have adverse effects on the cardiovascular system, contrary from what has been promoted by the tobacco industry[24]. The INTERHEART study, that recruited patients from 52 countries confirmed that no tobacco product can be considered as safe for cardiovascular health[59].

Patterns of tobacco consumption vary between regions, sex and social class, specially. A report from the National Institute of Health Doctor Ricardo Jorge showed that in Portugal tobacco was consumed daily or occasionally by 28.3% of the male population and by 16.4% of the female population, with higher prevalence in individuals aged between 25 and 34 years and lower in individuals between 65 and 75 years[60].

Average smokers are commonly leaner than non-smokers, however, oxidation of lipids and lower HDL-c were found more often in smokers that non-smokers[61]. Moreover, regular tobacco users have higher fasting blood glucose from lower insulin sensitivity and have usually higher blood pressures and heart rate. There is also a hypercoagulable and proinflammatory state characterised by the increase in fibrinogen levels, thromboxane A2, platelet activation, isoprostanes, and C-reactive protein[61,62]. Besides this, it is noticed an increase in carbon monoxide and a reduction in oxygen delivery to vital tissues which activates SNS and causes a vasoconstriction beyond the direct vascular effects of nicotine resulting in higher blood pressures[61,63].

1.2.5. Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is associated with increased risk of morbidity and mortality due to cardiovascular risk, in fact, there is a 2 to 4-fold increase in risk of incident coronary artery disease and ischemic stroke and a 1.5 to 3.6-fold increase in

mortality[64]. In 2015, 8.8% of people worldwide were estimated to have diabetes, in Portugal, at the same year, the prevalence was higher, 13.3% of the population had diabetes and more than 90% of whom had DMT2[65–67].

This disease is described as a complex progressive metabolic disorder characterized by impaired insulin secretion and/or action, associated with a progressive loss of islet β-cell and insulin resistance, respectively[68].

The pathophysiology behind CVD due to DMT2 is hyperglycaemia, insulin resistance or hyperinsulinemia, dyslipidaemia, inflammation, reactive oxygen species, endothelial dysfunction, hypercoagulability, and vascular calcification (**Figure 3**)[69]. The vascular dysfunction results in vasoconstriction contributing to hypertension[70].

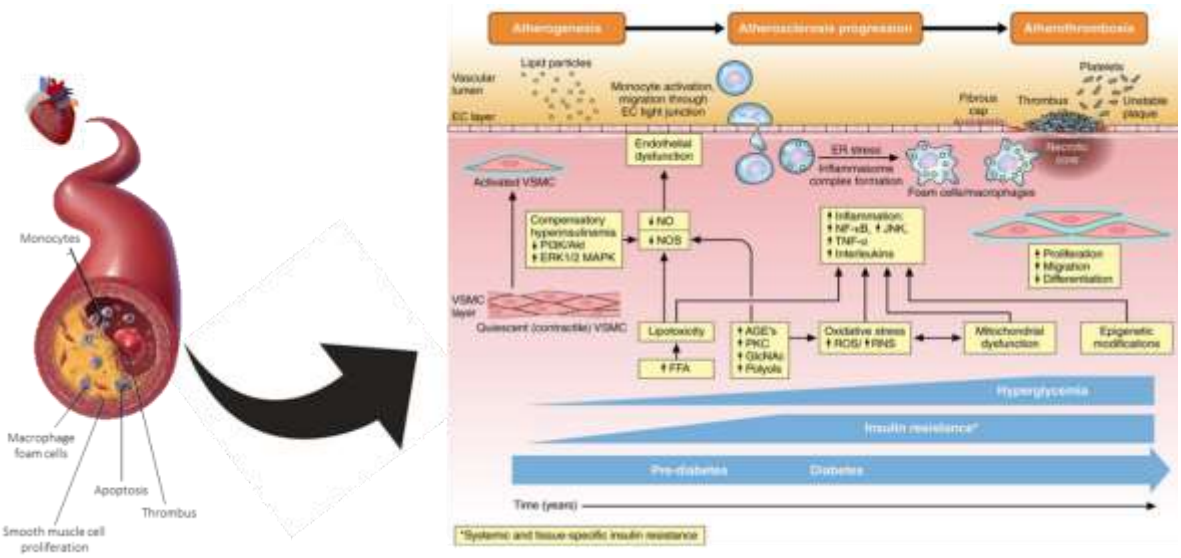

Figure 3 - Mechanism of CVD development from Diabetes. NO = nitric oxide; NOS = nitric oxide synthase; Akt = protein kinase B; ERK = extracellular signal-regulated kinase; GlcNAc = N-Acetylglucosamine; IL = interleukin; JNK = c-Jun N-terminal kinase; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K = phosphoinositide 3-kinase; RNS = reactive nitrogen species. Adapted from Rask-Madsen & King[71] and Wang *et al.*[69]

Therefore, it is important to monitor patients with DMT2 for cardiovascular risk and focus on a better glycaemic control trough lifestyle intervention and correct pharmacotherapy in order to reduce mortality rates[72,73].

1.2.6. Alcohol

In addition to smoking, alcohol use is another addictive habit that has influence in cardiovascular risk. In Portugal, recent data suggests that the annual average alcohol consumption is 12.4 L of pure alcohol in subjects with 15 or more years old. Beer (46%), wine (46%) and distilled spirits (8%) are the most common alcoholic beverages consumed by the Portuguese population[74].

The association between CVD and alcohol consumption is often described as J-shape or U-shape[75–79], meaning that while heavy drinking (8 or more drinks per week for woman and 15 or more drinks per week for men) or binge drinking (4 or more drinks during a single occasion for women and 5 or more drinks during a single occasion for men) is associated with a higher risk of CVD, light (any amount less than moderate drinking) to moderate (1 drink per day for women and 2 drinks per day for men) drinking has a protective effect comparing to abstainers[80].

However, a recent systematic analysis for the Global Burden of Disease 2016 of studies on alcohol use and burden for 195 countries and territories, displayed a significant contribution of alcohol to global death, disability, and ill health, concluding that the level of alcohol consumption that minimized harm across health outcomes was zero standard drinks per week[81]. Although there are several studies that indicate the existence of a J-shape or U-shape curve with cardioprotective benefits at low-to-moderate alcohol consumption, these effects are offset by the increased risk of other health-related harms, including cancer[81,82].

1.2.7. Cholesterol

As it was already mentioned, atherosclerosis is the landmark of CVD and it is essentially instigated by deposition of cholesterol and fibrous tissues in the arterial wall[83].

Studies have shown that the size of the particles matter, as HDL cholesterol levels are inversely associated with risk of CVD[84], while LDL cholesterol levels are positively associated with risk of major cardiovascular events[85].

It was initially hypothesised that dietary cholesterol contributes to the increase of serum cholesterol and thus, to the risk of heart disease, this resulted in recommendation

statements from AHA of limiting dietary cholesterol intake to 300 mg/d for healthy individuals[83]. Nevertheless, scientific evidence and experimental data did not validate that dietary cholesterol increases serum cholesterol, being verified that the increase intake of dietary cholesterol is associated with a decrease in endogenous *de novo* cholesterol synthesis, possibly as a homeostasis compensatory mechanism[83]. As a result of recent studies, AHA emitted a new statement in the 2015 -2020 Dietary guidelines for Americans indicating that cholesterol is not a nutrient of concern for overconsumption, however it still advises to eat as little as possible while consuming a healthy eating pattern[86].

Hence, new guidelines from AHA determine that if the patient with risk of cardiovascular disease has an altered lipid profile it is necessary to begin lipid-lowering pharmacotherapy besides healthy eating patterns in order to reduce the risk[87].

1.3. "Menos Sal Portugal" Project

As shown, different risk factors can be implicated in the pathophysiology of CVD, but there is one with major impact – Hypertension. Thus, because of the results of previous studies that indicate a higher salt consumption in Portuguese population and the impact of this overconsumption in blood pressure it was decided to create the project "*Menos Sal Portugal*". This project intends to access the impact of nutritional interventions in the risk reductions of hypertension and consequently CVD, which is imperative to demonstrate the importance of new health policies and to invest in nutritional education.

2. Aims

Different risk factors can be implicated in the pathophysiology of CVD, out of modifiable factors, hypertension appears to be the one with most impact. Healthy eating habits, including the reduction of salt consumption, has an important role in reducing the risk of hypertension and CVD. This study intends to evaluate the effectiveness of a salt reduction educational program versus a generic healthy lifestyle program in the population living in Lisbon metropolitan area.

The main aim of this study is to evaluate the impact of two different 12-week nutritional educational programs in the estimated salt consumption, assessed by 24-h urinary sodium excretion, in Portuguese adults.

The secondary aims consist in:

- Identifying the changes in blood pressure from baseline to week 12 after a nutritional intervention program focused on salt reduction in comparison to a general healthy lifestyle program;
- Identifying the changes in weight, BMI and waist circumference from baseline to week 12 after a nutritional intervention program focused on salt reduction in comparison to a general healthy lifestyle program;
- Identifying the changes in Mediterranean diet adherence, assessed by PREDIMED score, from baseline to week 12 after a nutritional intervention program focused on salt reduction in comparison to a general healthy lifestyle program.

3. Methods

3.1. Study design

This study was a consortium-initiated, randomized, blinded and parallel-controlled trial designed to assess the effectiveness of a salt reduction program versus a general healthy lifestyle program on salt intake reduction. The study was conducted in Lisbon, between January 2019 and August 2019, at CUF Descobertas and CUF Infante Santo.

3.2. Participants

Volunteers were recruited from Lisbon Metropolitan Area through social media, radio and television advertisement and were evaluated according to eligibility criteria (**Table 1**).

Table 1 - Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Adult men or women	Pregnant and breastfeeding women or women planning to become pregnant within the study period
Age 20-70 years	Subjects with current or previous cardiovascular disease (ischemic cardiovascular disease, angina stable or unstable; myocardial infarction, stroke or symptomatic peripheral arteriosclerosis)
Subjects with or without hypertension, not medicated or medicated with medication and diet stabilized for at least 3-weeks	Subjects with liver or kidney diseases or cancer
Subjects responsible for the purchase and confection of their meals	Subjects with history of drug, alcohol or other substances abuse, or other factors limiting their ability to cooperate during the study
Willing and able to comply with study protocol and sign informed consent	Subjects with especial dietary needs
	Health condition that compromise compliance with study requirements

All participants signed their written informed consent after receiving oral and written information about the study. The study protocol was approved by the Ethical Committee of Academia CUF Descobertas. The study is registered in ClinicalTrials.gov as NCT03830021.

3.3. Study protocol

Eligible volunteers were randomly allocated to one of the intervention groups using a computer-generated allocation sequence.

The study had the duration of 12 weeks and comprised five stages: an initial visit, followed by a run-in period of at least 1 week, and four additional visits with 4-weeks interval between each visit.

Participants were asked to collect a 24-h urine sample before visit 1 and visit 4. At visit 1 and 4, participants were also evaluated for anthropometric, blood pressure measurements and PREDIMED score.

Additionally, participants had three individual educational sessions of approximately 30 min, at visit 1, 2 and 3, led by two registered nutritionists. Weekly calls were performed between the visits to reinforce the information transmitted during the sessions and to clarify eventual questions or doubts.

3.4. Intervention

3.4.1. Salt-reduction Program

During the first session, participants were informed about the salt consumption in the Portuguese population and how it exceeds WHO recommendations. It was explained the effects of salt overconsumption in health and which foods they should avoid in order to reduce salt intake.

For the second session, it was explained how they could interpret food labels and how to choose foods with lower salt content belonging to the same group. Herbs and spices were also addressed in this session as an important salt alternative and it was explained how to consume and take the most advantages of these ingredients.

Finally, in session 3 it was explained how to interpret salt nutritional claims and the importance of achieving the recommend intake of fruits and vegetables. Additionally, it

was discussed the effect of potassium, calcium and magnesium on blood pressure and it was indicated the best food sources of these minerals.

Participants received a flyer after each session regarding these topics. To facilitate the access to the information provided, presentations slides and flyers where sent by email in the end of the visit day.

Participants of the salt reduction program also received two practical educational sessions between visit 1 and 2, visit 2 and 3, and visit 3 and 4. These sessions were performed in supermarkets (Pingo Doce®) where the same topics of the previous sessions were discussed in a more practical way, with tips and demonstrations. During these sessions, participants could also expose their daily difficulties when it comes to healthy food choices with a lower salt content.

3.4.2. Healthy Lifestyle Program

Throughout the first session, it was discussed the impact of the Mediterranean diet in health, especially cardiovascular health. Each food group of this food guide was carefully analysed, indicating the best food choices and which foods to avoid as a part of the principles of the Mediterranean food pattern.

In session 2, different lifestyle topics were addressed, starting with the importance of hydration and how to increase water intake during the day. Physical activity was also discussed during this session. It was indicated the levels of physical activity in the adult Portuguese population, explained the importance of exercise and how to become more active. Lastly, sleep quality was also a discussed topic in the second session, especially the effects of a bad quality sleep in health, indicating the number of recommended sleep hours and how to improve sleep quality.

Session 3 focussed on the negative health impact of addictive habits, specifically alcohol consumption and smoking habits. Healthy culinary methods were also addressed, indicating how to reduce nutritional losses and carcinogenic compounds synthesis during food preparation. The final topic was food waste in terms of the reality in Portugal and how to reduce domestic food waste.

Participants received a flyer after each session regarding the addressed topics. To facilitate the access to the information provided, the sessions and flyers were sent by email in the end of the visit day.

3.5. Urinary sodium excretion

The 24-h urinary sodium excretion was used to estimate the daily sodium intake. The 24-h urinary sodium excretion reflects about 90% of the sodium consumed from all food sources[88].

Participants were orally and written instructed for 24-h urine sampling. Participants were instructed to discard the first morning urine specimen and to collect, thereafter, all the urine specimens into the container provided, including the first morning urine specimen of the following day. Participants were instructed to store the urine samples at 4 °C, during the 24h of urine collection.

Sodium in urine were analysed at *Centro de Medicina Laboratorial Germano de Sousa*. The estimated salt intake was derived from 24-h urinary sodium excretion as 1 mmol/24h sodium = 0.05844 g/d salt[42].

3.6. Blood Pressure measurements

Measurements of heart rate[89], systolic and diastolic blood pressure[89] were performed according to *Direção-Geral da Saúde* protocol.

Blood pressure measurements were performed in a quiet environment at room temperature. Participants should not have smoked, consumed stimulants, such as alcohol and coffee, and exercised in the last hour. In the first visit two measurements were performed on each arm with an interval of 2 minutes between each measure in order to identify the dominant arm. In subsequent visits, blood pressure measurements were performed twice in the dominant arm with an interval of 2 minutes. One more measure was performed if there was a great discrepancy between previous measures[89].

3.7. Anthropometric measurements

Volunteers were measured according to protocol from *Direção-Geral da Saúde* for body weight[90], height[90] and waist circumference[90].

3.7.1. Height

Height measurement was performed using a stadiometer (SECA®). Participants were asked to remove accessories that could interfere with assessment.

Briefly, participants should be positioned vertically with arms along the body and the palm of their hands faced to the body. The head needed to be in the horizontal plan of Frankfort and eyes looking forward. Two sequenced measurements were performed using the calliper with a sensitivity of 0.1 cm[90].

3.7.2. Body weight

Participants were asked to remove their shoes and all their heavy belongings, such as belt, jacket, watch, scarf, jewellery, cell phone, etc. Afterwards, participants were invited to step on a calibrated scale (Tanita® model UM-076) with their feet positioned parallel and arms positioned along the body with the palm of their hands faced to the body. The head needed to be straight and the participant should be looking straight ward. Two sequenced measurements were performed with sensitivity of 100 g. If the difference between measurements was superior to 0.5 kg, one more measure needed to be performed[90].

3.7.3. Waist circumference

Participants should be positioned vertically with arms along the body with the palm of their hands faced to the body and feet together to equally distribute body weight. Waist circumference was measured using a measurement tape (SECA®) in the straightest abdominal area after a normal expiration. Two sequenced measurements were performed with a sensitivity of 0.1 cm. If the difference between measurements was superior to 1 cm, one more measure needed to be performed[90].

3.8. Questionnaires

At the initial visit, a questionnaire was administered to the participants to collect some socio-demographic and health information: sex, race, date of birth, educational level,

work situation, diagnosis of hypertension, diagnosis of diabetes, diagnosis of dyslipidaemia and current medication.

PREDIMED questionnaire was administered by registered nutritionists, at visit 1 and 4, to evaluate the adherence to the Mediterranean dietary pattern[91].

3.9. Outcomes

The primary outcome was a reduction in estimated salt intake assessed by 24-h urinary sodium excretion, from baseline to the end of follow-up (12 weeks). The secondary outcomes were changes in systolic and diastolic blood pressure, changes in weight, BMI and abdominal circumference and changes in the PREDIMED score from baseline to 12 weeks.

3.10. Statistical analysis

Statistical analysis was performed using SPSS V.25 software. Differences were considered statistically significant when $p<0.05$.

The Skewness and Kurtosis tests were used to test normality of the distribution. Wilcoxon signed-rank test was used to compare the differences between baseline and post-intervention period. Changes during 12-week intervention period, between the two intervention groups, were compared by Mann-Whitney test.

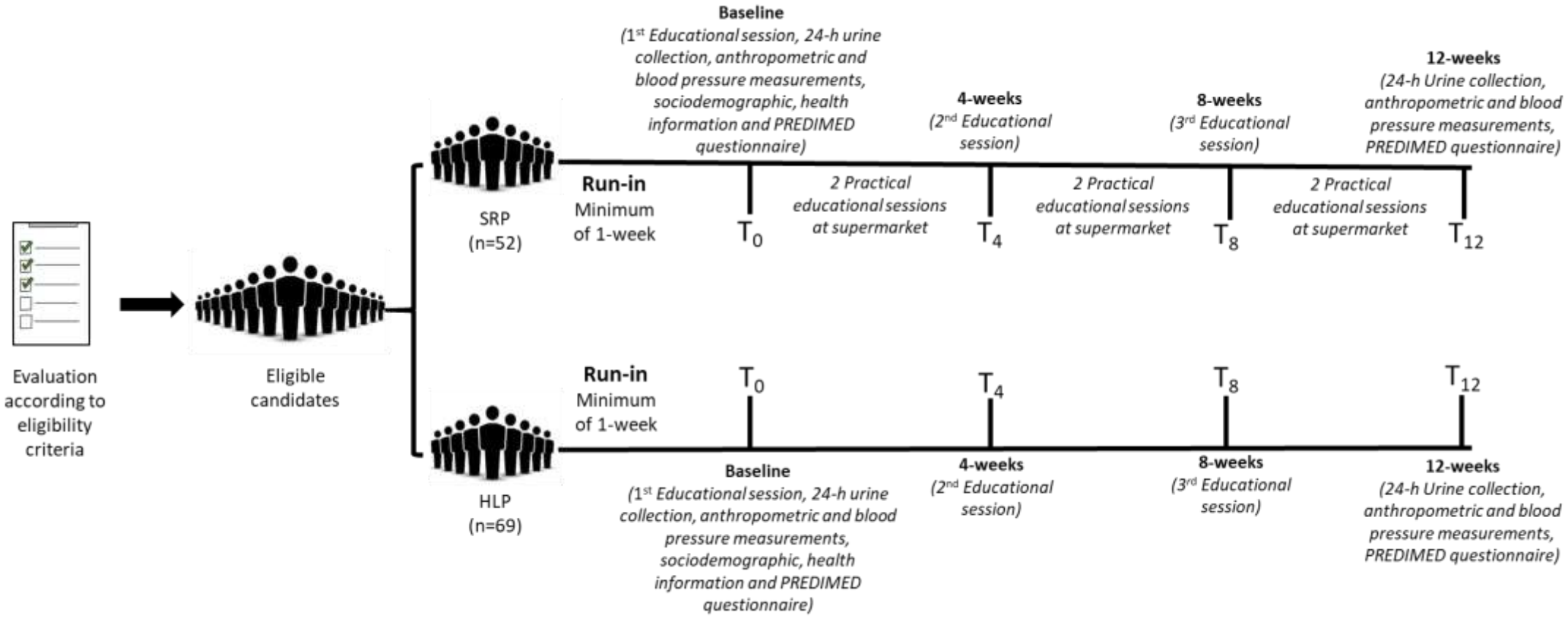

Figure 4 – Study design. SRP = Salt-reduction Program; HLP = Healthy Lifestyle Program

4. Results

Between March 28 and August 31 of 2019, 300 individuals enrolled the study. This study will present the preliminary results of 121 participants that completed the study until the end of July. From these 121 participants, 52 were randomly allocated to the Salt-reduction Program Group and 69 to the Healthy Lifestyle Program Group. Baseline characteristics were similar between groups, apart from sex (**Table 2**). Nevertheless, differences within the primary and secondary outcomes were independent of sex ($p>0.05$).

Table 2 – Baseline characteristics

	Salt-reduction Program Group (n=52)	**Healthy Lifestyle Program Group (n=69)**
Sex		
Male	20 (38.5%)	15 (21.7%)
Female	32 (61.5%)	54 (78.3%)
Age (years)	44.8 (22-69)	46.8 (20-68)
Race		
Caucasian	51 (98.1%)	68 (98.6%)
Other	1 (1.9%)	1 (1.4%)
Scholarity level		
High school level or CTESP and below	13 (25.0%)	24 (34.8%)
Bachelor´s degree or higher	39 (75.0%)	45 (65.2%)
Employment status		
Unemployment	1 (1.9%)	7 (10.1%)
No paid employment	7 (13.5%)	11 (15.9%)
Full or part-time employment	44 (84.6%)	51 (73.9%)
Hypertension	8 (15.4%)	12 (17.4%)
Dyslipidaemia	11 (21.2%)	25 (36.2%)
T2DM	0 (0.0%)	1 (1.4%)
Antihypertensive drugs	8 (15.4%)	10 (14.5%)
Lipid-lowering drugs	5 (9.6%)	10 (14.5%)
Oral antidiabetic drugs	1 (1.9%)	1 (1.4%)

Data are expressed as n (%). Age is expressed as mean (minimum-maximum). CTESP = Higher Professional Technical Courses. *p< 0.05 between Salt-reduction Program and Healthy Lifestyle Program

At baseline, mean estimated salt intake (n=117) was 7.04 ± 2.67 g/d for the Salt-reduction Program and 6.88 ± 2.70 g/d for the Healthy Lifestyle Program. After the programs, mean estimated salt intake was 7.02 ± 2.97 g/d for the Salt-reduction Program and 6.11 ± 3.34 g/d for the Healthy Lifestyle Program. Thus, the estimated salt intake had an average reduction of 0.02 ± 3.78 g/d in Salt-reduction Program and 0.77 ± 2.90 g/d in the Healthy Lifestyle Program. Estimated salt intake reduction was only significant in Healthy Lifestyle Program ($p<0.05$; **Figure 5**).

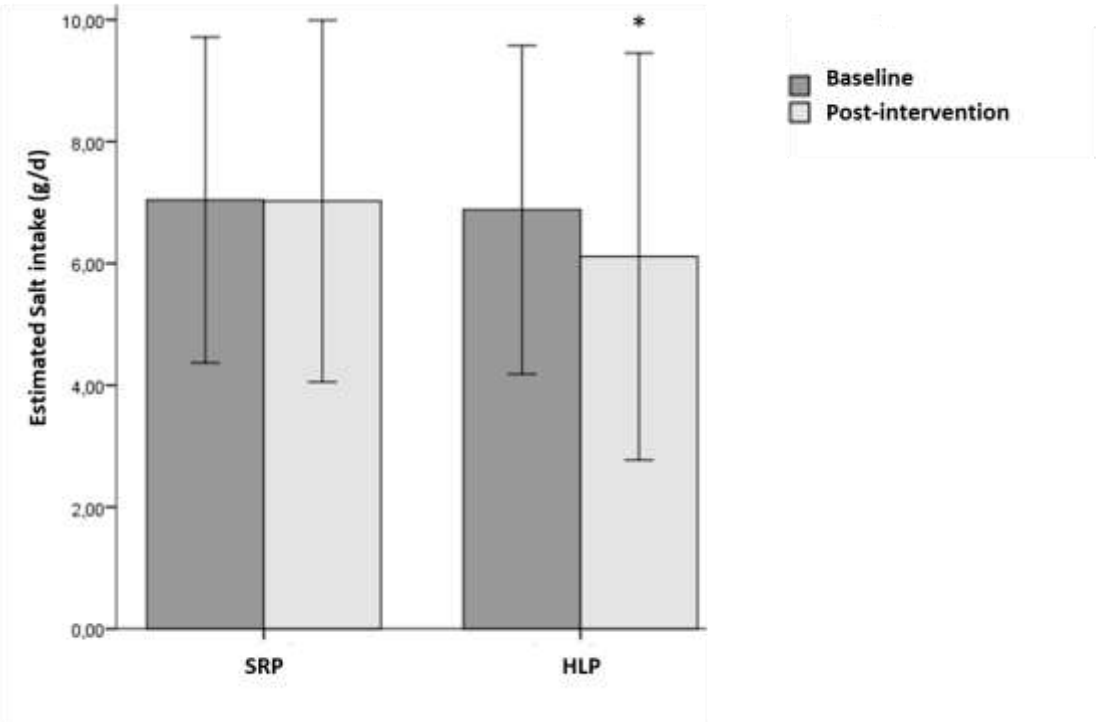

Figure 5 – Estimated salt intake at baseline and post-interventions. SRP = Salt-reduction Program; HLP = Healthy Lifestyle program. *p<0.05 vs baseline

For secondary outcomes, it was observed a tendency in reduction of systolic blood pressure and heart rate after the intervention, in both groups, especially in the Salt-reduction Program, although without statistical significance ($p>0.05$; **Table 3**).

Both Salt-reduction Program and Healthy Lifestyle Program Group caused a significant reduction in body weight, with a higher average reduction in the Healthy Lifestyle Program Group (from 72.9 ± 15.0 to 72.1 ± 14.7 kg; $p<0.05$; **Table 3**). Similar results were recorded for BMI.

No changes were observed in classes of BMI of individuals under 65 years old between baseline and after 12 weeks of both interventions (p=0.919). At baseline, 41.4% of participants had normal weight, 33.3% were overweight and 25.2% had obesity. After the intervention, the percentages were 44.1%, 31.5% and 24.3%, respectively. For the elderly group (n=10), no changes were seen from baseline to the end of interventions (p=1.00); 70% had normal BMI and 30% were overweight according to the BMI classification of this age group.

Moreover, significant differences were seen in the PREDIMED score after both interventions. Average score increased from 8.0 ± 2 to 9.0 ± 2 in the two groups (p<0.05; **Table 3**). PREDIMED score was grouped into high-adherence (score≥10) or low-adherence to the Mediterranean diet. Results from qui-square test showed that the proportion of participants with high adherence increased significantly after both dietary interventions (p<0.05; **Figure 6**).

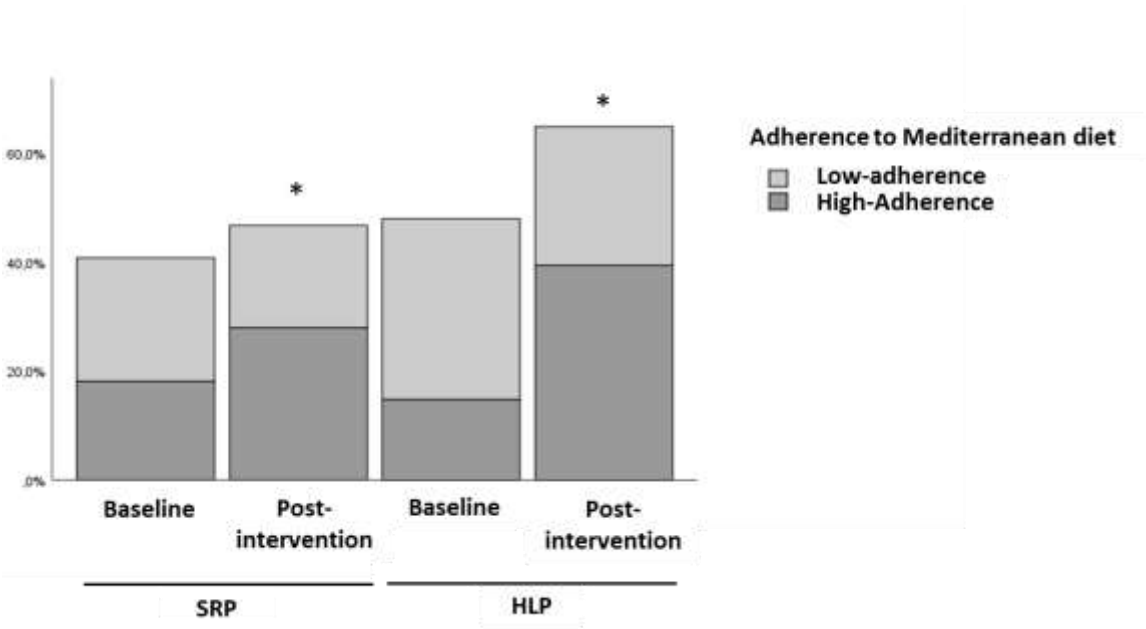

Figure 6 – Adherence to the Mediterranean diet. SRP = Salt-reduction Program; HLP = Healthy Lifestyle program. *p<0.05 vs baseline

Nevertheless, the proportion of participants eating 2 or more servings of vegetables per day and 3 or more servings of fruits per day increased significantly only in the Healthy Lifestyle Program (p<0.05; **Table 4**).

Participants were divided in two groups, according to the bodyweight loss - participants who loss less than 1 kg *vs* those who loss 1 kg or more after interventions. Bodyweight loss was independent from sex (p=0.440) and from the intervention group (p=0.814) where participants were initially allocated.

The group with higher weight loss had a mean reduction of 2.51 kg and the group with lower weight loss had a mean increase of 0.50 kg in bodyweight (p<0.05; **Table 5**). Higher PREDIMED score variation was associated with higher bodyweight loss (equal or superior to 1 kg). Participants that lost at least 1 kg had a significant reduction of waist circumference, systolic blood pressure and diastolic blood pressure (p<0.05; **Table 5**), in comparison to those that did not achieve this bodyweight loss.

Additionally, bodyweight loss was superior in participants diagnosed with dyslipidaemia (-1.28 ± 2.37 kg) comparing to those without the disease (-0.45 ± 1.97 kg) (p<0.05; **Figure 7**).

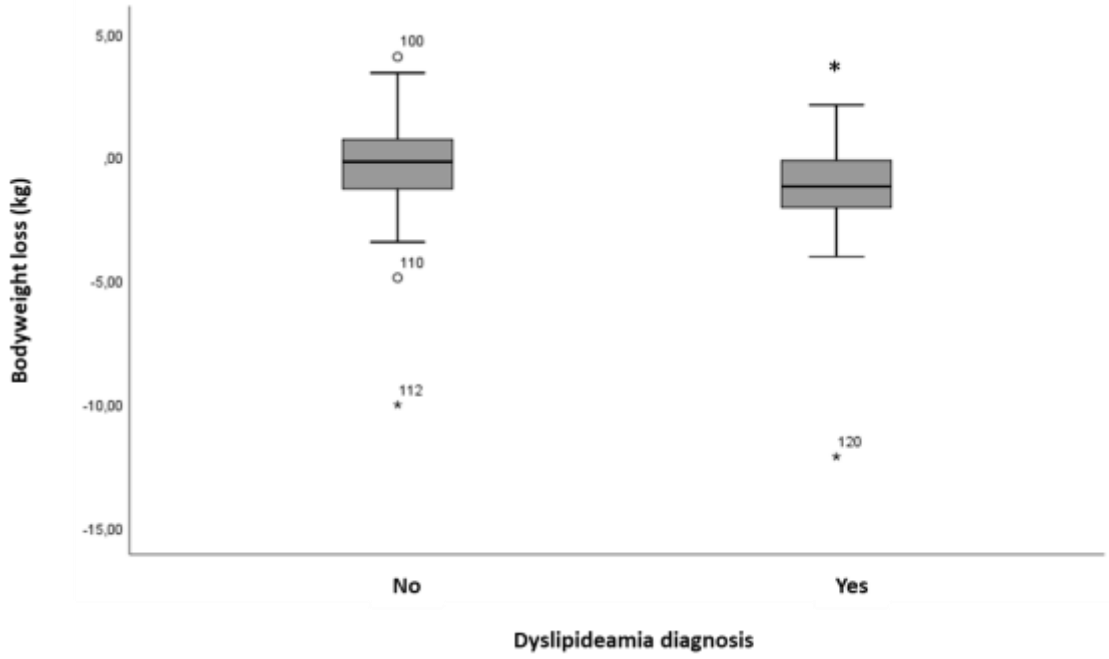

Figure 7 – Bodyweight loss according to dyslipidaemia diagnosis. *p<0.05 vs participants without dyslipidaemia diagnosis

Table 3 – Secondary outcomes

	Salt-reduction Program (n=52)				Healthy Lifestyle Program (n=69)			
	Baseline	Post-intervention	p value	Change (95% Cl)	Baseline	Post-intervention	p value	Change (95% Cl)
Bodyweight (kg)	76.9 ± 16.6	76.3 ± 16.9	0.014*	-0.57 (-0.99 to -0.15)	72.9 ± 15.0	72.1 ± 14.7	0.009*	-0.80 (-1.39 to -0.20)
BMI (kg/m^2)	27.2 ± 5.3	27.0 ± 5.3	0.013*	-0.20 (-0.36 to -0.04)	27.3 ± 5.3	27.0 ± 5.2	0.013*	-0.23 (-0.49 to 0.03)
Waist circumference (cm)	85.4 ± 13.2	85.4 ± 13.5	0.964	-0.07 (-0.84 to 0.71)	85.7 ± 14.3	84.6 ± 13.0	0.57	-1.11 (-2.20 to -0.03)
Systolic blood pressure (mmHg)	115.8 ± 15.1	113.4 ± 14.0	0.102	-2.36 (-5.07 to 0.35)	114.1 ± 15.9	112.6 ± 15.6	0.460	-1.45 (-4.27 to 1.38)
Diastolic blood pressure (mmHg)	73.7 ± 9.1	74.0 ± 9.0	0.555	0.34 (-1.08 to 1.75)	75.5 ± 9.1	74.8 ± 8.6	0.615	-0.69 (-1.93 to 0.54)
Heart rate (bpm)	71.7 ± 12.4	69.0 ± 9.0	0.072	-2.66 (-5.20 to -0.13)	74.1 ± 10.1	73.7 ± 8.9	0.998	-0.34 (-2.44 to 1.76)
PREDIMED score	8.0 ± 2.0	9.0 ± 2.0	0.005*	1.00 (0.00 to 1.00)	8.0 ± 2.0	9.0 ± 2.0	<0.001*	1.0 (1.00-1.00)

Data are in mean ± standard deviation. *p<0.05 vs baseline. No statistical significance in the variation of all the measurements between groups

Table 4 – Food groups consumption evaluated by PREDIMED questionnaire

	Salt-reduction Program (n=52)		p value	Healthy Lifestyle Program (n=69)		p value
	Baseline	Post-intervention		Baseline	Post-intervention	
Vegetables (≥2 portions/d)	23 (44.2%)	32 (61.5%)	0.077	16 (23.2%)	28 (40.6%)	0.028*
Fruit (≥3 portions/d)	21 (40.4%)	29 (55.8%)	0.116	32 (46.4%)	45 (65.2%)	0.026*
Legumes (≥3 portions/week)	13 (25.0%)	18 (34.6%)	0.185	19 (27.5%)	28 (40.6%)	0.369
Fish and seafood (≥3 portions/week)	34 (65.4%)	34 (65.4%)	1.000	42 (60.9%)	47 (68.1%)	0.586
Red meat (<1 portion/d)	35 (67.3%)	41 (78.8%)	0.284	55 (79.7%)	59 (85.5%)	0.106
Butter, margarine and cream (<1 portion/d)	36 (69.2%)	35 (67.3%)	0.833	45 (65.2%)	48 (69.6%)	0.586
Pastries (<3 portions/week)	38 (73.1%)	35 (67.3%)	0.520	47 (68.1%)	56 (81.2%)	0.374
Nuts (≥3 portions/week)	24 (46.2%)	32 (61.5%)	0.116	31 (44.9%)	36 (52.2%)	0.394
Sugary drinks - Soft drinks and juices (<1 drink/d)	47 (90.4%)	50 (96.1%)	0.240	62 (89.9%)	65 (94.2%)	0.346
Wine (≥7/week)	5 (9.6%)	4 (7.8%)	0.727	11 (15.9%)	6 (8.7%)	0.195
Olive oil (≥4 spoons/d)	10 (19.2%)	10 (19.2%)	1.000	13 (18.8%)	9 (13.0%)	0.352

Data are expressed as n (%). *p< 0.05 vs baseline

Table 5 – Bodyweight loss and variation in outcomes

	Bodyweight loss < 1 kg (95% CL) n=73	**Bodyweight loss ≥ 1 kg (95% CL) n=48**	**p value**
Bodyweight variation (kg)	0.50 (0.25 to 0.75)	-2.51 (-3.10 to -1.93)	<0.001*
Waist perimeter variation (cm)	-0.14 (-0.94 to 0.66)	-1.46 (-2.74 to -0.18)	0.019*
Systolic blood pressure variation (mmHg)	0.17 (-1.95 to 2.30)	-4.90 (-8.57 to 1.22)	0.017*
Diastolic blood pressure variation (mmHg)	0.89 (-0.16 to 1.93)	-1.97 (-3.59 to -0.36)	0.007*
Heart rate variation (bpm)	-1.73 (-3.76 to 0.29)	-0.74 (-3.47 to 1.98)	0.609
Variation of estimated salt intake (g/d)	-0.19 (-0.95 to 0.56)	-0.86 (-1.90 to 0.18)	0.297
PREDIMED score variation	0.55 (0.16 to 0.94)	1.25 (0.71 to 1.79)	0.023*

Data are in mean. *p<0.05 vs bodyweight loss inferior to 1 kg

5. Discussion

In the present study, we intended to evaluate the impact of two dietary educational programs (Salt-reduction Program and Healthy Lifestyle Program) in risk factors of CVD. Both groups decreased bodyweight and increased adherence to the Mediterranean diet. It was also observed a reduction of estimated salt intake 0 in the Healthy Lifestyle Program and blood pressure reductions in the individuals that loss more weight.

Various studies have demonstrated a low adherence of the Portuguese population to the Mediterranean diet [32,92,93], which reinforces the importance of the implementation of dietary programs to increase the adherence to this healthy dietary pattern in order to prevent CVD. According to Afonso *et al.*, a high adherence to the Mediterranean diet is defined by a PREDIMED score≥10[91]. Our findings indicate that both 12-week dietary interventions increased the Mediterranean diet adherence evaluated by the PREDIMED score, but the score remained lower than 10. Nevertheless, the proportion of participants with high adherence to the Mediterranean diet (score ≥10) increased after both dietary education programs. Similar results were seen after dietary programs in studies from Logan *et al.*[94], Zazpe *et al.*[95], Sahingoz and Dogan[96] and Philippou *et al.*[97].

Even though Mediterranean diet was not the focus of the Salt-reduction Program, during the educational sessions it was recommended the use of herb and spices as substitutes to salt, the reduction of processed foods and the increased intake of fruits and vegetables. These recommendations are all principles of the Mediterranean diet[33], which justifies the increase in the PREDIMED score of the participants from this group.

Also, as seen in the study of Bonaccio *et al.,* nutrition knowledge is associated with higher adherence to the Mediterranean diet[98]. Despite, most of the participants of this study had higher education and paid work which are characteristics associated with higher nutrition knowledge[99], the adherence of the participants to the Mediterranean diet was low at baseline. In the present study, we intended to empower subjects to make healthier choices, increasing the nutritional knowledge of the participants which resulted in higher adherence to the Mediterranean diet after both interventions.

The weight loss and consequently the decrease in BMI observed in both intervention groups can be associated with this increased adherence to the Mediterranean diet as seen in the review of Dinu *et al*.[100]. If we focus on the meta-analysis of Esposito *et al*.[101] increase adherence to Mediterranean diet results in a mean weight loss of 1.75 kg. In the present study the weight loss was lower: 0.57 kg in the Salt-reduction Program and 0.80 kg in the Healthy Lifestyle Program. This difference may be due to the different methodologies used to evaluate Mediterranean diet adherence. Most of the studies in this meta-analysis assessed Mediterranean diet by 24-h food recalls, 3-d food diaries or food frequency questionnaire whereas we evaluated using the PREDIMED score[101].

The study from Estruch *et al.* that used the same methodology but considered a high adherence to the Mediterranean diet when the score≥9, observed that increase in Mediterranean diet adherence did not resulted in significant bodyweight loss[102]. However, the effects of the Mediterranean diet were compared to a low-fat diet instead to the participant's usual dietary habits.

Reaching a normal bodyweight is essential to reduce CVD risk. As mentioned previously, atherosclerosis is common in several CVD and the underlying cause of atherosclerosis is chronic inflammation[103]. If there is an excessive accumulation of body fat mass, the quantity and/or size of adipocytes increases. These changes may result in adipose tissue dysfunction, increasing the production of pro-inflammatory cytokines which can induce insulin resistance, thus contributing to CVD[104]. Weight loss results in shrinkage of adipocytes, which consequently reduces the production of pro-inflammatory cytokines and increases the production of anti-inflammatory cytokines, especially interleukin-10[104,105].

Unfortunately, due to blood collection-related constraints, it was not possible to evaluate the cardiometabolic and inflammatory profile of the participants of the study. However, a better metabolic profile would be expected after weight loss.

At baseline, 33.3% and 25.2% of our study population were respectively, overweight and obese which are in accordance with the results from the First Portuguese Health Examination Survey[26]. Although both intervention programs resulted in BMI reduction, the decrease was not enough to change the class of BMI into which subjects were

classified, i.e. they remained classified as overweight or obese. Nonetheless, with BMI it was not possible to infer about participants lean and fat mass and to characterize their body fat mass distribution, a significant factor in metabolic health risk. Therefore, to minimize these limitations, waist circumference was also evaluated[106].

While there was no significantly reduction in waist circumference after both interventions, the mean waist circumference was under the cut-off that determines central obesity and thus higher cardiovascular risk[51]. In addition, subjects with higher weight loss had significantly higher reduction of waist circumference which results in CVD risk reduction. These results were similar to the study from Rothberg *et al*.[107] after a weight management program.

Subjects with dyslipidaemia diagnosis lost more weight comparing to those without the diagnosis. This disease is traditionally associated with unhealthy dietary habits, therefore the recommendations given in the intervention programs appear to have more impact in the subjects with dyslipidaemia[108]. A study of Michishita *et al.*[109] also observed a reduction on bodyweight after a dietary program and a program focused on exercise in individuals with dyslipidaemia, reinforcing the role of lifestyle interventions not only in the prevention of dyslipidaemia but also in the treatment of the disease as weight loss is highly importance in triglycerides and LDL-c control[108].

Higher weight loss also resulted in significant reductions of both systolic and diastolic blood pressure. Similar results were observed in the prospective study of Sabaka *et al.*[110] and after a weight loss intervention study of Fazliana *et al.*[111]. In the study of Neter *et al.*, blood pressure reduced 1.05 mmHg systolic and 0.92 mmHg diastolic per each kg of bodyweight loss[112]. However, we observed that the mean reduction of bodyweight in the group that loss ≥1 kg was 2.51 kg, and this resulted in a mean decrease of 4.90 mmHg in systolic and 1.97 mmHg in diastolic blood pressure, which is a higher decrease in the systolic blood pressure than expected. These results suggest that there are other factors that may have contributed to decrease in blood pressure apart from weight loss. The group that reduced more weight also had higher increase adherence to Mediterranean diet. Mediterranean diet is associated with higher intake of fruits and vegetables, whole cereals, nuts and olive oil that together guarantees an adequate intake of beta carotene, vitamin C, tocopherols, linolenic acid, various important minerals, and several possibly

beneficial non-nutrient substances such as polyphenols and anthocyanins exhibiting, according to Kokkinos *et al.*, beneficial impact in blood pressure[113]. Doménech *et al.* evaluated the changes in ambulatory blood pressure after a Mediterranean diet supplemented with extra-olive oil and a Mediterranean diet supplemented with nuts. The authors observed that the reduction in systolic blood pressure after the interventions were 3.14 mmHg and 2.35 mmHg, respectively[114].

Contrarily to what was expected, a reduction in salt consumption and systolic blood pressure was not observed in the Salt-reduction Program. Since we recommended the reduction of salt intake either by addition during confection or by consumption of processed foods and recommended the increase ingestion of foods rich in potassium, calcium and magnesium, we could expect a reduction on these parameters[115]. It is important to remember that we did not test salt sensitivity, meaning that some participants probably did not have salt sensitivity, and thus do not respond to salt reduction[116].

Estimated salt intake reduction was only reduced in the Healthy Lifestyle Program. This reduction can be associated with the increased intake of fruits and vegetables after the Program. These food groups are rich in micronutrients such as potassium which has an important effect on blood pressure[117]. Higher potassium intake results in decreased sodium excretion by directly increasing natriuresis, decreasing renin release, reducing proliferation and migration of smooth muscle cells, reducing oxidative stress and oxidized LDL, reducing plaque aggregation and improving endothelium-dependent vasodilatation[117].

In the Salt-reduction Program, the stimulus for the consumption of fruits and vegetables was only explored in the last educational session of the program, not giving enough time for participants to change these dietary habits in time to verify differences in potassium intake and consequently in sodium excretion.

In both groups estimated salt intake was above WHO recommendations[43], the estimated average salt intake values were closer to the results from the first Portuguese Health Examination Survey (7.3 g/d)[26] than the PHYSA study (10.7 g/d)[42].

Estimated salt intake results needs to be interpreted very carefully since this study does not reflect the complete sample recruited in the project and because even though the methodology used to estimate the daily sodium intake was the 24-h urinary sodium excretion, it was not considered the 24-h urinary creatinine/kg of weight formula to exclude urine samples outside the reference ranges[42] as it would reduce significatively the number of samples analysed. Samples were only excluded if the total volume was inferior to 500 ml.

6. Conclusion

Nutrition has an important role in the prevention of CVD through reduction of risk factors such as obesity and hypertension. In order to achieve healthier dietary habits, it is important to empower subjects to make conscious dietary choices. This can be accomplished by dietary educational programs performed by registered nutritionists which are experts in the field.

Results from this study demonstrate that a dietary program specifically designed to reduce salt intake may not be effective to reduce sodium consumption. A lifestyle educational program promoting Mediterranean diet, which has been proven to be beneficial in CVD risk reduction, comprehend other factors that contribute to sodium homeostasis beyond salt intake, such as the consumption of vegetables and fruits which are rich in potassium.

Comprehensive dietary interventions may therefore have a promising effect in the improvement of dietary habits specifically in the consumption of vegetables and fruits that are below the recommended intake of WHO (5 servings), in Portugal.

The increase in Mediterranean diet adherence resulted in bodyweight reduction and consequently, lower blood pressure in the subjects that loss more weight. These parameters are all related to hypertension and consequently, CVD risk.

The outcomes of this study are in accordance to the results of recent investigations that show the importance of dietary habits in the reduction of mortality rates. Thus, it is essential to invest in nutrition field by targeting these current highly prevalent diseases not only in an individual-level, increasing nutritional monitoring in health centres and hospitals, but also in a community-level, creating new campaigns, educational programs and health policies, which in the long term will reduce significantly the costs in public health.

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