“EFFECTS OF A WEIGHT LOSS PROGRAM ON CARDIOVASCULAR RISK IN ADULTS WITH OBESITY: THE WLM3P STUDY”

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A dissertation submitted in partial fulfilment of the requirements for the Degree of Masters in Metabolism and Human Nutrition at Faculdade de Ciências Médicas | NOVA Medical School of NOVA University Lisbon

March, 2023

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ACKNOWLEDGMENTS

The whole journey to the conclusion of this thesis had several highs and lows. The challenge was sometimes enormous but always encouraged by people I am extremely thankful for.

I would like to express a special thanks to my supervisor Dra. Vanessa Pereira and my colleague Alexandra Salvador Pisco. They have always been a huge support for me, both from a technical and scientific point of view and also from a personal point of view.

I also want to thank all the support of the supervisor Dra. Diana Teixeira. She was always available and motivated me to see solutions when faced with difficult scenarios.

Thanks to supervisor Dra. Filipa Cortez, I had the opportunity to collaborate in a clinical study of my current professional practice.

Last but not least, I would like to thank Filipe, who was always supportive, a good listener, and never failed to cheer me up.

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ABBREVIATIONS

CVD - Cardiovascular Disease

HDL-C - High-Density Lipoprotein Cholesterol

WL – Weight Loss

TG – Triglycerides

LDL-C - Low-Density Lipoprotein

VLDL - Very Low-Density Lipoprotein

SCORE 2 - Systemic Coronary Risk Estimation 2

TC – Total Cholesterol

LCD - Low-Carbohydrate Diets

WLM3P - Weight Loss Maintenance 3 Phases Program

BMI - Body Mass Index

TRE - Time-Restricted Eating

DER - Total Daily Energy Requirements

TEI – Total Energy Intake

CHO – Carbohydrate

PROT – Protein

SAT - Saturated

sdLDL - Small Dense Low-Density Lipoprotein-Cholesterol

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ABSTRACT

Background: Obesity increases cardiovascular risk through factors such as increased fasting plasma triglycerides and low high-density lipoprotein cholesterol, which represents metabolic manifestations of adiposopathy. Commercial weight loss programs that include interventions with evidence of efficacy in clinical trials have been considered effective alternatives for body weight management and to reduce the cardiovascular risk associated with obesity. In this regard, the Weight Loss Maintenance 3 Phases Program is a protocol treatment for overweight adults, which assumes a high-protein and low-carbohydrate diet, weekly consultations with a nutritionist, use of food supplements, motivational support, time-restricted eating, high-protein specific food and online platform monitoring, which makes it different from the standard low-carbohydrate approach.

Objective: The aims were to evaluate the impact of the Weight Loss Maintenance 3 Phases Program on lipid profile (triglycerides, high-density lipoprotein cholesterol, and triglycerides/high-density lipoprotein cholesterol ratio), blood pressure, the cardiovascular risk with Systematic COronary Risk Evaluation2 and the correlation between nutritional intake and lipid profile at baseline and at 6 months, compared to a low-carbohydrate diet.

Methodology: A total of 112 participants with obesity (Body Mass Index ≥ 30 kg/m^2 and ≤ 39.9 kg/m^2) were randomly assigned to the Weight Loss Maintenance 3 Phases Program or low-carbohydrate diet.

Results: At 6 months the Weight Loss Maintenance 3 Phases Program resulted in a more pronounced increase in high-density lipoprotein cholesterol (mg/dL) compared to the low-carbohydrate diet [+7.9 (8.2) vs +4.9 (6.2); p=0.046]. The triglycerides/high-density lipoprotein cholesterol ratio at 6 months improved in both groups compared to baseline but without a statistical difference (p=0.267). No statistical differences were found in other outcomes between groups except weight loss and diastolic blood pressure (p<0.001 and p<0.021 respectively). The Weight Loss Maintenance 3 Phases Program had a lower total energy intake (kcal per day) [1313.8 (1167.5|1406.8) vs 1444.4 (1304.6|1573.6); p<0.001], a lower carbohydrate intake (% of total energy intake) [16.7 (14.4|24.6) vs 25.1 (22.1|28.6); p<0.001], a higher protein intake (% of total energy intake) [31.8 (28.6|34.2) vs 27.0 (24.6|29.4); p<0.001] and also higher fiber intake (g per day) [19.2 (15.5|21.3) vs 15.1 (12.9 to 18.8); p=0.040], compared to low-carbohydrate diet at 6 months. A positive correlation between triglycerides (r=0.38; p=0.011) and protein intake at 6 months in the low-carbohydrate diet and a positive correlation between triglycerides (r=0.53; p<0.001) and fiber intake at 6 months in the Weight Loss Maintenance 3 Phases Program were found.

Conclusion: Our data showed that the Weight Loss Maintenance 3 Phases Program was more effective in increasing high-density lipoprotein cholesterol than a low-carbohydrate diet after 6 months of intervention for weight loss.

KEYWORDS

Nutrition; Behavioural Intervention; Obesity; Weight Loss; Health Promotion; Cardiovascular Risk

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RESUMO

Fundamentação: A obesidade aumenta o risco cardiovascular por meio de fatores como o aumento dos triglicerídeos plasmáticos e diminuição do colesterol das lipoproteínas de alta densidade em jejum, que representam manifestações metabólicas da adiposopatia. Programas comerciais de perda de peso que incluem intervenções com evidências de eficácia em ensaios clínicos têm sido considerados alternativas eficazes para o controlo do peso corporal e para a redução do risco cardiovascular associado à obesidade. Neste sentido, o *Weight Loss Maintenance 3 Phases Program* é um protocolo de tratamento para adultos com obesidade, que pressupõe uma dieta rica em proteínas e baixo teor de hidratos de carbono, consultas semanais com nutricionista, uso de suplementos alimentares, apoio motivacional, alimentação com restrição de tempo, alimentos específicos hiperproteicos e plataforma on-line de monitorização, o que o torna diferente da abordagem padrão de dieta de baixo teor de hidratos de carbono.

Objetivos: Os objetivos foram avaliar o impacto do *Weight Loss Maintenance 3 Phases Program* no perfil lipídico (triglicerídeos, colesterol de lipoproteínas de alta densidade, no rácio triglicerídeos/colesterol de lipoproteínas de alta densidade), pressão arterial, o risco cardiovascular com Avaliação Sistemática de Risco COronário2 e a correlação entre ingestão nutricional e perfil lipídico no início e aos 6 meses, em comparação com uma dieta de baixo teor de hidratos de carbono.

Metodologia: Um total de 112 participantes com obesidade (Índice de Massa Corporal ≥ 30 kg/m^2 e ≤ 39,9 kg/m^2) foram aleatoriamente designados para o *Weight Loss Maintenance 3 Phases Program* ou dieta com baixo teor de hidratos de carbono.

Resultados: Aos 6 meses, o *Weight Loss Maintenance 3 Phases Program* resultou em um aumento mais pronunciado no colesterol de lipoproteínas de alta densidade (mg/dL) em comparação com a dieta de baixo teor de hidratos de carbono [+7,9 (8,2) vs +4,9 (6,2); p=0,046]. O rácio triglicerídeos/colesterol de lipoproteínas de alta densidade aos 6 meses melhorou em ambos os grupos em comparação com a início, mas sem diferença estatística (p = 0,267). Nenhuma diferença estatística foi encontrada em outros resultados entre os grupos exceto na perda de peso e na pressão arterial diastólica (p<0.001 e p<0.021, respetivamente). O *Weight Loss Maintenance 3 Phases Program* teve uma menor ingestão total de energia (kcal por dia) [1313.8 (1167.5 |1406.8) vs 1444.4 (1304.6|1573.6); p<0,001], uma menor ingestão de hidratos de carbono (% da ingestão total de energia) [16.7 (14.4|24.6) vs 25.1 (22.1|28.6); p<0,001], uma maior ingestão de proteína (% da ingestão total de energia) [31.8 (28.6 |34.2) vs 27.0 (24.6|29.4)); p<0,001] e também maior ingestão de fibras (g por dia) [19.2 (15.5|21.3) vs 15.1 (12.9 to 18.8); p=0,040], em comparação com dieta de baixo teor de hidratos de carbono aos 6 meses. Uma correlação positiva entre triglicerídeos (r=0,38; p=0,011) e ingestão de proteína aos 6 meses na dieta de baixo teor de hidratos de carbono e uma correlação positiva entre triglicerídeos (r=0,53; p<0,001) e ingestão de fibras aos 6 meses na *Weight Loss Maintenance 3 Phases Program* foram encontrados.

Conclusão: Os nossos dados mostraram que o *Weight Loss Maintenance 3 Phases Program* foi mais eficaz em aumentar o colesterol de lipoproteínas de alta densidade em comparação com uma dieta de baixo teor de hidratos de carbono após 6 meses de intervenção para perda de peso.

PALAVRAS-CHAVE

Nutrição; Intervenção Comportamental; Obesidade; Perda de Peso; Promoção de Saúde; Risco Cardiovascular

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INTRODUCTION

Obesity is an adiposity-based chronic, progressive, and relapsing disease. The incorporation of the characteristics of "adiposity" includes the total amount, distribution, and function of adipose tissue. This terminology may ultimately help improve the International Classification of Diseases, based upon three dimensions: etiology, degree of obesity, and health risks[1].

Obesity negatively affects both physical and psychological health, with a higher risk of developing type 2 diabetes, cardiovascular disease (CVD), osteoarthritis, some types of cancer, dementia, and Alzheimer's disease[2].

According to data from the National Health Survey with Physical Examination, from 2015, obesity affected around 2.5 million adults (a prevalence of around 28.7%)[3] and CVD is the main cause of mortality, being the cause of around 31.9% of deaths in Portugal[4].

Adiposopathy (or "sick fat") is defined as pathologic adipose tissue anatomic/functional disturbances promoted by a positive caloric balance in genetically and environmentally susceptible individuals that result in adverse endocrine and immune responses that may directly promote CVD and may cause or worsen the metabolic disease. Blood lipid levels are established risk factors for non-communicable diseases, including obesity, some of which have shown causal relationships with various cardiovascular diseases. Adiposity-associated dyslipidemia is characterized by low high-density lipoprotein cholesterol (HDL-C) levels and an increase in triglyceride-rich lipoproteins. A 5-10% weight loss (WL) is associated with significant reductions in triglycerides (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-C), and an increase of HDL-[5,6].

Although the guideline recommends the reduction of levels of LDL-C in a way to reduce cardiovascular risk, after his reduction, a high residual CVD risk still exists due to other lipid components, such as TG and HDL-C[7,8]. Several studies have reported that the combination of high TG and low HDL-C levels was a predictor of CVD independent of LDL-C level[9–11].

Triglyceride concentrations are an important predictor of CVD[12], despite the direct mechanism of TG and CVD pathophysiology remains unclear. TG-rich lipoproteins (very low-density lipoprotein (VLDL), chylomicrons, and their remnants) have atherogenic properties, and thus management of TG concentrations is a key aspect of metabolic health[13]. Elevated TG concentrations are strongly associated with visceral adiposity, insulin resistance, CVD, and other indicators of metabolic dysfunction due to excess adipose tissue mass[14]. Hypertriglyceridemia promotes the exchange of TG from VLDL for cholesterol esters from LDL-C and HDL-C particles, creating small, lipid-poor particles. Small HDL-C particles are more susceptible to degradation, thus contributing to the low-HDL-C concentrations often observed in individuals with obesity[14]. There are no treatment goals for triglycerides, but <150 mg/dL is considered to indicate lower risk, whereas higher levels indicate a need to look for other risk factors[15].

The studies indicate that individuals with primary low HDL-C levels have higher risks of CVD than individuals with optimal lipid profiles[16]. The INTERHEART study showed that individuals with abnormal blood lipid levels were 3 times more likely to have CVD than those with normal blood lipid levels[17]. Plasma TG concentration has also been

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suggested as an important biomarker in predicting WL[18,19]. Rader DJ and colleagues. showed that elevated HDL-C levels can reduce certain cardiovascular risk factors[20] and in epidemiological studies, HDL-C levels are inversely related to the risk of CVD[21]. Although patients with hyperlipidaemia can control their blood lipid levels with drugs, more than 50% of patients cannot receive drug treatment due to side effects, financial constraints, or other reasons[22]. The optimal metabolic metrics for adults are defined as HDL-C ≥40 and 50 mg/dL for males and females, respectively[23].

Moreover, combining the two lipid measures into one as the ratio of TG to HDL-C has been proven to be a reliable early biomarker of insulin resistance[24,25], which is strongly associated with CVD[26,27]. The TG/HDL-C ratio, a parameter calculated from the standard lipid profile, is more accessible than insulin resistance in real-world clinical practice. The logarithm of the TG/HDL-C ratio is also widely used to assess the plasma atherogenicity and is known as the atherogenic index of plasma for a strong correlation with LDL-C particles and increased fractional esterification rate for cholesterol in plasma[28,29]. The TG/HDL-C ratio is also associated with several cardiometabolic diseases, including obesity[30] and values of ≥ 2.5 (women) and ≥ 3.5 (men) provide useful cut-points[31]. Although the benefits of lowering the TG/HDL-C ratio remain unclear, elevated TG levels and low HDL-C levels are regarded as markers of residual cardiovascular risk beyond LDL-C[32].

The need to estimate total cardiovascular risk in apparently healthy individuals has since 1994 been strongly advocated by the joint recommendations from The European Society of Cardiology, European Society of Hypertension, European Atherosclerosis Society and other societies. The Systemic Coronary Risk Estimation 2 (SCORE2) algorithm used in the guidelines 2021 European Society of Cardiology, estimates an individual's 10-year risk of fatal and non-fatal CVD events (myocardial infarction, stroke) in apparently healthy people aged 40-69 years with risk factors that are untreated or have been stable for several years. SCORE2 is calibrated to four clusters of countries (low, moderate, high, and very high CVD risk) that are grouped based on national CVD mortality rates published by the WHO and Portugal is considered a moderate-risk country (107.9 CVD deaths per 100 000 population)[33]. The benefits of using SCORE2 are a reliable estimation of age and sex-specific relative risks, adapted risk prediction models to the circumstances of each European region, an intuitive, easy-to-use tool, takes account of the multifactorial nature of CVD, calculation of the 10-year risk of fatal and nonfatal cardiovascular disease events, allows flexibility in management, allows a more objective assessment of risk over time, establishes common language of risk for clinicians and shows how risk increases with age[33]. In general, risk factor treatment recommendations are based on categories of CVD risk ('low-to-moderate', 'high', and 'very high'). The cut-off risk levels for these categories are numerically different for various age groups to avoid undertreatment in the young and to avoid overtreatment in older persons (low–moderate risk: SCORE2 <2.5% under age 50, SCORE2 <5% ages 50–69; high risk: SCORE2 2.5–7.5% under age 50, SCORE2 5–10% ages 50–69; very high risk: SCORE2 >7.5% under age 50; SCORE2 >10% ages 50–69) (Table1)[15]. The SCORE2 was calculated using individual values of age, sex, current smoking, systolic blood pressure, total cholesterol (TC) and HDL-C[15].

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Table 1. Cardiovascular disease risk categories based on SCORE2 in apparently healthy people according to age

	<50 years	50–69 years
Low-to-moderate CVD risk	<2.5%	<5%
High CVD risk	2.5 to <7.5%	5 to <10%
Very high CVD risk	≥7.5%	≥10%

CVD = cardiovascular disease

More recent studies have indicated that WL is often similar to comparison diets in the long term, but low-carbohydrate diets (LCD) may have advantages in reducing TG, while increasing HDL-C[34–42], including interventions lasting less than 6 months[18]. Although part of this benefit may be due to the greater WL with the LCD, the mechanism underlying the significant increase in the HDL-C level of subjects undergoing this intervention is still not clear, and more research on the underlying mechanism is needed. Of clinical significance an increase in the HDL-C levels was generally considered beneficial, equal to a 7.45% reduction in the relative risk of CVD[18]. A high intake of saturated fat is associated with dyslipidaemia and an increased risk of CVD, as well as overweight and obesity. However clinical trials investigating the effects of an LCD have not produced enough sufficient data to support this concern[43].

Dietary modifications, together with an increase in physical activity and reduction of inactivity, are the first-line therapies to improve lipid profile. However, more ambitious targets in the area of dietetic management of obesity may be advisable for those who are at a higher risk of cardiovascular and metabolic complications[20]. Nutritional recommendations for WL should consider the negative energy balance, the overall quantity and quality of the diet, the number and timing of meals and the distribution of macronutrients throughout the day in a way to reduce cardiovascular risk. These recommendations should ensure long-term compliance[44–46].

An overweight treatment program has three main objectives: to decrease body weight, reduce the risk of long-term associated comorbidities such as cardiovascular risk, and prevent the regain of lost weight[45,47].

Commercial WL programs that include sustainable behavioural change interventions and that demonstrate evidence of efficacy in clinical trials have been considered effective alternatives for body weight management and to reduce the cardiovascular risk associated with obesity[48,49]. Despite the availability of multiple WL programs (Jenny Craig, Weight Watchers, Nutrisystem, Health Management Resources, Medifast, OPTIFAST, SlimFast, The Biggest Loser Club and others), little is known about the impact on lipid profile[39,50].

However, no clinical study has yet been carried out to demonstrate if the Weight Loss Maintenance 3 Phases Program (WLM3P) produces cardiovascular health benefits. The present study aims to analyse the effect of WLM3F on TG, HDL-C, TG/HDL-C ratio improvement and cardiovascular risk through SCORE2, compared to a standard low-carbohydrate diet (LCD) in non-diabetic adults with obesity [body mass index (BMI) ≥30 kg/m^2].

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AIMS

- Evaluate the impact of the WLM3P on lipid profile (TG, HDL-C and TG/HDL-C ratio) after 6 months of intervention with the WLM3P in adults with obesity (Body Mass Index (BMI) ≥ 30 kg/m^2 and ≤ 39.9 kg/m^2), compared to LCD;
- Assess changes in the cardiovascular risk (SCORE2) after 6 months of intervention with the WLM3P in adults with obesity (BMI ≥ 30 kg/m^2 and ≤ 39.9 kg/m^2), compared to LCD;
- Assess changes in the lipid profile (TG, HDL-C and TG/HDL-C ratio) considering nutritional intake after 6 months of intervention with the WLM3P in adults with obesity (BMI ≥ 30 kg/m^2 and ≤ 39.9 kg/m^2), compared to LCD.

OUTCOMES

- Difference between the intervention group and the control group regarding TG, HDL-C and TG / HDL-C ratio at baseline and 6 months;
- Difference between the intervention group and the control group regarding nutritional intake at baseline and 6 months;
- Difference between the intervention group and the control group regarding cardiovascular risk (SCORE2) at baseline and 6 months;
- Correlation between weight loss and variation of TG, HDL-C and TG/HDL-C ratio between the intervention group and control group at baseline and at 6 months;
- Correlation between nutritional intake and TG, HDL-C and TG/HDL-C ratio between the intervention group and control group at baseline and 6 months.

METHODOLOGY

The WLM3P, developed by nutritionists in 2006, is a protocol treatment for overweight adults, which assumes a high protein low-carbohydrate diet, weekly consultations with a nutritionist, use of food supplements, motivational support, time-restricted eating 14:10 (14 hours of metabolic fasting and 10 hours duration of eating), high-protein specific food and online platform monitoring (web app), which makes it different from the standard low-carbohydrate approach.

The current study was part of an ongoing research project directed by Dr. Vanessa Pereira, PhD student at Nova Medical School. This study was supervised by PhD Conceição Calhau and is entitled “Pilot Study: Effectiveness of Nutritional Interventions in Adults with Obesity”. Approved by Ethics Committee nº108/2018/CEFCM (Attachment 1).

STUDY DESIGN

The WLM3P study was a randomized controlled trial, single-blinded, comprising an 18 months (6 months WL period followed by a 12 months weight maintenance period) study conducted at NOVA Medical School, NOVA University of

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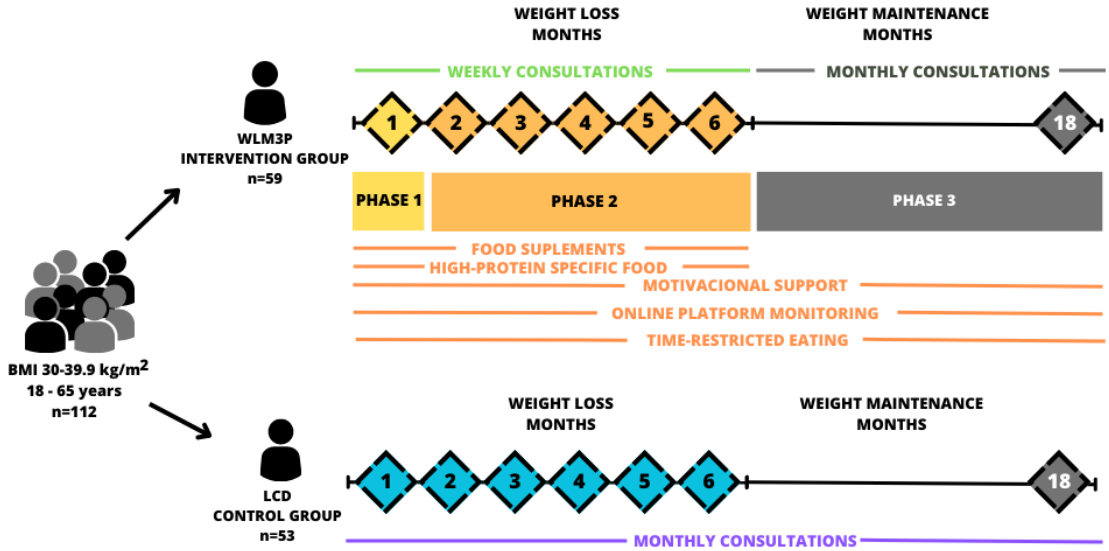
Lisbon, between March 2020 and January 2023. Participants were allocated randomly to two groups: an intervention group (WLM3P) and a control group (LCD). The participants were blinded to the presence of the two intervention arms.

A total of 112 generally healthy, nondiabetic with obesity adults (BMI: 30–39,9 kg/m^2) were randomly assigned in equal proportions in a parallel-design weight-loss diet study: WLM3P (n=59) or LCD (n=53) (Figure 1). Enrolment for the first participant in the first cohort started in April 2020 and follow-up for the last participant was completed in January 2023. Randomization was performed to assign eligible participants to an intervention or control group, according to an automated computer-generated randomization scheme, (sequentially numbered) that was controlled by the principal investigator, who was not involved in recruitment and intervention delivery.



BMI – Body Mass Index; WLM3P - Weight Loss Maintenance 3 Phases Program; LCD – Low-Carbohydrate Diet; BMI – Body Mass Index

Figure 1. Study protocol

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PARTICIPANTS

Participants with a BMI between 30.0 kg/m^2 and 39.9 kg/m^2 and aged between 18 and 65 years. All participants were available to adhere to a WL intervention, comply with the study protocol, and sign the respective informed consent (Attachment 2). Table 2 shows the inclusion and exclusion criteria of the participants.

Table 2. Participant's inclusion and exclusion criteria

Inclusion criteria
Individuals were eligible to participate if they meet the following eligibility criteria:
• BMI ≥ 30 kg/m^2 and ≤ 39.9 kg/m^2, non-diabetic;
• Interested in being enrolled in a WL intervention;
• Available to comply with the study protocol and sign the protocol informed consent.
Exclusion criteria
• Pregnant, breastfeeding or planning to become pregnant within the study period;
• Celiac disease or other intestinal diseases;
• Hormonal or thyroid pathology (untreated hypothyroidism);
• Type 1 or type 2 diabetes mellitus;
• Severe heart failure and/or Pacemaker carrier;
• Altered blood clotting;
• Renal impairment and urinary incontinence;
• Chronic liver disease other than non-alcoholic hepatic steatosis;
• Autoimmune diseases and/or chronic use of corticosteroids;
• Use of weight loss medication/other nutritional supplements;
• Allergy or intolerance to any component of prescribed supplements;
• Psychiatric illness (e.g. use of more than 2 antidepressants, major depression, bipolar disorder);
• Subjects prescribed with 5 or more drugs;
• Surgery or hospitalization in the last 30 days;
• A previous attempt to lose weight in the last month and/or WL of more than 10 kg in the previous 3 months before the start of the study or who have had bariatric surgery;
• Excessive alcohol consumption (self-reported: drinking more than 3 glasses of wine/day or equivalent) or drug addiction;
• History of eating disorders (diagnosis of anorexia nervosa, bulimia nervosa or purging disorder);
• Plans to undertake long-term travel in the forthcoming 18 months;
• Vegetarianism or the need for other specific diets;
• Insufficient Portuguese language skills to complete the study questionnaires.

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INTERVENTION STRATEGY

The WLM3P consists of a nutritional intervention for the treatment of overweight and obesity, based on scientific evidence, created and developed in Portugal, in 2006, by nutritionists. The program is divided into three phases, each with specific objectives and characteristics. The first two phases are designed for weight loss and include weekly nutrition sessions. The third phase, which is not included in this study, focuses on weight maintenance and includes monthly nutrition sessions. Additionally, the WLM3P includes a high-protein specific food, time-restricted eating (TRE), motivational support (coaching), weekly consultations, food supplements and online platform monitoring (mobile and web app).

The diet followed in the program primarily consists of raw and cooked non-starchy, low-carb vegetables without restriction, and included seafood, lean meats and poultry, eggs, tofu, avocado, olives, and low sugar dairy products (e.g., milk, yoghurt, cheese, kefir, calcium-fortified soy/nuts milk), nuts (almonds, walnuts, and hazelnuts), seeds, oats, low-carb wheat bread (10g of carbs/slice of bread), and high fiber fruit (i.e., berries). Fruits with a higher glycaemic index were gradually introduced. Allowed drinks include water, flavored carbonated water without sugar, infusion tea, coffee, and herbal extracts. Table 3 describes the dietary prescription in WLM3P intervention. This WLM3P aims to decrease body weight by reducing fat mass, improving lipid profile, reducing the risk of long-term associated comorbidities and preventing weight regain.

Table 3. Dietary prescription in WLM3P intervention

Characteristics	**Phase 1**	**Phase 2**
	Weight loss	**Weight loss**
Follow-up frequency	Weekly	Weekly
Duration	1 month	5 months
Energy restriction	70% DER	70% DER
Carbohydrates (%)	10-15% TEI	15%-20% TEI
Protein (%)	40-45% TEI	35-40% TEI
Lipids (%)	35-45% TEI	35-40% TEI
Fiber, g/day	20	20-25

DER - Total daily energy requirements; TEI – Total energy intake

In addition to these characteristics, the WLM3P uses dietary supplements, such as a vitamin and mineral supplement with fructo-oligosaccharides and galacto-oligosaccharides, a liver support supplement with silymarin, a diuretic supplement with extracts of green tea, L-carnitine, bromelain, horse chestnut, birch, cherry, blackcurrant, dandelion, meadowsweet, vine, potassium, rutin and also high in vitamins C and E and a WL enhancer with *glycine max*, soy (lecithin), L-carnitine, apple pectin, garcinia cambogia dry extract and chromium. The dietary supplements aim to compensate for restrictions and enhance weight loss, as described in the document approved by the Ethics Committee (nº108/2018/CEFCM).

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CONTROL GROUP

Participants randomized to an active control group received a healthy standard LCD divided into two periods (WL period of 6 months and weight maintenance period of 12 months). Weight maintenance is not included in this study. The characteristics of each phase are indicated in Table 4. In the LCD group, no high-protein specific food or food supplements were used, as described in the document approved by the Ethics Committee (nº.108/2018/CEFCM). In the LCD group, nutrition sessions were performed monthly. The diet allowed vegetables, meat and poultry, fish and seafood, nuts and seeds, fruits, unsweetened dairy products (plain whole milk and plain Greek yogurt) and extra virgin olive oil. The LCD dietary prescription included also recommendations such as:

- Avoid all sugars and sweeteners such as white sugar, brown sugar, honey, corn syrup, maple syrup;
- Avoid all artificial sweeteners such as aspartame, except stevia;
- Limit starchy foods;
- Use olive oil for cooking;
- Avoid using vegetable seed oils such as canola oil;
- Avoid deep fried food.

Table 4. Dietary prescription in LCD intervention

Characteristics	**Phase 1** **Weight loss**
Follow-up frequency	Monthly
Energy restriction	70% DER
Duration	6 months
Carbohydrates (%)	≤26% TEI
Protein (%)	35-40% TEI
Lipids (%)	≤35% TEI
Fiber, g/day	20-25

DER - Total daily energy requirements; TEI – Total energy intake

DIETARY ASSESSMENT

Dietary intake was assessed at baseline and 6 months intervention, using food diaries filled by the participants, complete with photographs of the meals referring to 3 days. The food diaries were then analysed by trained dietitians (the investigators), using the "*Manual Fotográfico de Quantificação de Alimentos IAN-AF 2015-2016*"[51] for assessment of portions and a database, built by the investigators with nutritional information based on the Portuguese Nutrition Database[52], United States Department of Agriculture Nutrient Database[53] and, when relevant, with the nutritional information on the labels of the food products consumed by the participants.

BLOOD SAMPLES, LIPID PROFILE AND CARDIOVASCULAR RISK

Fasting venous blood samples were collected for laboratory measurements: HDL-C and TG. Blood sampling was performed at the baseline and 6 months of the trial and collected during the morning (07:00–11:00) after an overnight fast of 10-12 hours. Cardiovascular risk was assessed using the TG/HDL-C ratio and SCORE2. The optimal metabolic metrics for adults are defined as HDL-C ≥40 or 50 mg/dL for males and females, respectively[23] and the TG/HDL-C ratio

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cut-off points considered were ≥2.5 for females and ≥3.5 for male[31]. The HeartScore® (risk assessment and management program) was used to obtain SCORE2 data (classification in categories according to Table 1) considering age, smoking status, systolic blood pressure and total cholesterol, LDL-C and HDL-C.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation. Differences were considered statistically significant when $p<0.05$. Categorical variables were described through absolute (n) and relative (%) frequencies, while continuous variables were described as mean and standard deviation, or median, interquartile (IQR) range, and minimum and maximum, when appropriate. Data were tested for normality by performing the Kolmogorov–Smirnov test and analysing the distribution using histograms. A comparison of variables in the same group (baseline vs. 6 months) was performed using parametric tests (Student's t-test) and nonparametric tests (Wilcoxon test), as appropriate, considering normality assumptions. For between-groups (WLM3P vs. LCD) comparisons, parametric tests (Student's t-test) and nonparametric tests (Mann-Whitney) were used as appropriate, considering normality assumptions. The outcomes as dependent variables and as independent (explanatory) variables regarding the compared groups (WLM3P vs LCD), adjusted for age, sex, baseline IMC, and baseline glucose (used since differences were observed at baseline) at 6 months. Coefficient regression (beta) and 95% confidence intervals (95% CI) are presented. The significance level used was 0.05. Statistical analysis was performed using the Statistical Package for the Social Sciences version 29.

RESULTS

A total of 112 adults with obesity (81 women, 31 men), and a BMI of 34±2.4kg/m^2, were randomized in WLM3P (n=59) and LCD (n=53). Three participants (5.1%) in the WLM3P group and 1 participant (1.9%) in the LCD group reported taking medications for hypercholesterolemia.

At baseline, there are no statistical differences between the groups according to HDL-C (p=0.136), TG (p=0.276) and TG/HDL-C ratio (p=0.841). In addition, 81% of the WLM3P participants and 79.2% of the LCD participants had TG< 150 mg/dL. The TG/HDL-C ratio with a baseline value of >2.5 in female participants was 19.5% (n=8) in the WLM3P group and 38.5% (n=15) in the LCD group, without a statistically significant difference (p=0.462). The TG/HDL-C ratio with a value >3.5 at baseline of male participants was 47.1% (n=8) in WLM3P and 35.7% (n=5) in LCD, without statistically significant difference (p=0.905). Table 5 indicates the baseline characteristics of study participants, including HDL-C and TG/HDL-C ratio stratified by gender.

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Table 5. Baseline characteristics of study participants

Characteristics		WLM3P n=59	LCD n=53	*P* value
Age (years), mean (sd)		44.0 (8.8)	46.2 (8.4)	0.176[2]
	18–33; n (%)	8 (13.6)	6 (11.3)	
	34–49; n (%)	31 (52.5)	28 (52.8)	0.931[1]
	50–65; n (%)	20 (33.9)	19 (35.8)	
Gender, n (%)				0.777
	♀	42 (71.2)	39 (73.6)	
	♂	17 (28.8)	14 (26.4)	
BMI (kg/m2), mean (sd)		33.9 (2.6)	34.1 (2.2)	0.852[2]
Weight (kg), mean (sd)		95.5 (11.5)	95.4 (11.9)	0.940[2]
Glucose (mg/dL), mean (sd)		83 (8.6)	88.1 (10.3)	**0.008[2]**
Smoking status, n (%)				0.691[1]
	Never smoked	32 (54.2)	33 (62.3)	
	Ex-smoker	23 (39.0)	17 (32.1)	
	Active smoker	4 (6.8)	3 (5.7)	
Reported medications for hypercholesterolemia, n (%)		3 (5.1)	1 (1.9)	0.620[2]
Hypertension, n (%)		7 (11.9)	12 (22.6)	0.129[1]
Dyslipidaemia, n (%)		3 (5.1)	3 (5.7)	0.161[1]
Blood Pressure (mm Hg), mean (sd)				
	Systolic	123.5 (14.4)	123.4 (10.9)	0.948[1]
	Diastolic	84.4 (9.4)	84.7 (9.0)	0.904[1]

Characteristics		WLM3P n=59	LCD n=53	*P* value
HDL-C (mg/dL), mean (sd)		50.2 (12.6)	53.8 (13.3)	0.136[2]
	♀ >50 mg/dL, n (%)	25 (61)	30 (76.9)	0.316[1]
	♀ <50 mg/dL, n (%)	16 (39)	9 (23.1)	
	♂ >40 mg/dL, n (%)	9 (52.9)	8 (57.1)	0.229[1]
	♂ <40 mg/dL, n (%)	8 (47.1)	6 (42.9)	
LDL-C (mg/dL), mean (sd)		112.5 (34.6)	118.6 (28.7)	0.318[2]
	<115, n (%)	34 (57.6)	28 (52.8)	0.489[1]
	≥115, n (%)	25 (42.4)	25 (47.2)	
TG (mg/dL), median (P25-P75)		102 (77-135)	115 (83-143)	0.276[3]
	<150, n (%)	47 (81)	42 (79.2)	0.813[1]
	>150, n (%)	11 (19)	11 (20.8)	
TG/HDL-C ratio, median (P25-P75)		2.5 (1.6-3.0)	2.2 (1.6-3.1)	0.841[3]
	♀ > 2.5, n (%)	8 (19.5)	15 (38.5)	0.462[1]
	♀ < 2.5, n (%)	33 (80.5)	24 (61.5)	
	♂ > 3.5, n (%)	8 (47.1)	5 (35.7)	0.905[1]
	♂ < 3.5, n (%)	9 (52.9)	9 (64.3)	

[1]Chi-Square test; [2]T-test for independent samples; [3]Mann Whitney test; WLM3P - Weight Loss Maintenance 3 Phases Program; LCD -low-carbohydrate diet; ♀ - female gender; ♂ - male gender; HDL-C - high-density lipoprotein cholesterol; TG – triglycerides

At baseline, there were no statistical differences between the groups according to SCORE2 (p=0.779). The low-to-moderate CVD risk was 76.3% (n=45) in WLM3P and 83% (n=44) in LCD; the high CVD risk was 23.7% (n=14) in WLM3P and 17% (n=9) in LCD group; and there weren't participants with very high CVD risk (Table 6).

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Table 6. Baseline SCORE2 categories of study participants

	WLM3P n=59	LCD n=53	*P* value
Low-to-moderate CVD risk, n (%)	45 (76.3%)	44 (83%)	0.779[1]
High CVD risk, n (%)	14 (23.7%)	9 (17%)	

[1]Chi-Square test; CVD- Cardiovascular Disease

At 6 months, the WLM3P had a more pronounced increase in HDL-C (mg/dL), compared to LCD [+7.9 (8.2) vs + 4.9 (6.2); p=0.046]. At 6 months, 93.5% of WLM3P participants and 93.3% of LCD participants showed TG<150mg/dL and 100% of participants in WLM3P and 93.5% in LCD reduced ≥5% of initial weight (p=0.078). The diastolic blood pressure (mm Hg) reduced more at the WLM3P compared to LCD [-7.5 (-11.5|-4.0) vs -6.0 (-9.0|-2.9); p= 0.021]. The TG/HDL-C ratio was reduced at 6 months compared to baseline in both groups but without a statistical difference (p=0.267). The TG/HDL-C ratio with a value >2.5 in female participants was 3% (n=1) in WLM3P and 12.5% (n=4) in LCD, without a statistically significant difference between groups (p=0.394). The TG/HDL-C ratio >3.5 in male participants was 15.4% (n=2) in WLM3P and 7.7% (n=1) in LCD, without statistically significant difference between groups (p=0.246). A total of 93.4% of the participants had an optimal TG level (<150mg/dL) at 6 months. Table 7 shows changes in body weight and lipid profile between groups, including HDL-C and TG/HDL-C with stratification by gender at 6 months compared to baseline.

Table 7. Changes in body weight and lipid profile between WLM3P and LCD groups at 6 months compared to baseline

Characteristics	**WLM3P**		**LCD**		**Difference**	**95% CI**	***P* value**
	n		n				
Δ Body Weight (kg), mean ±sd	46	-18 (5.7)	46	-11.5 (6.5)	-6.9	-- 9.3 to - 4.5	**<0.001[1]**
Δ Body Weight Loss (%), mean ±sd	46	19.0 (5.2)	46	11.9 (6.1)	-5.6	--9.8 to -1.7	**<0.001[1]**
Δ Systolic Blood Pressure (mm Hg), median (P25|P75)	46	-9.5 (-19.5 | -2)	46	-8.3 (-13.9 |-0.5)	-2.3	- 6.7 to 2.1	0.306[3]
Δ Diastolic Blood Pressure (mm Hg), median (P25|P75)	46	-7.5 (-11.5| -4)	46	-6.0 (-9.0|-2.9)	-3.1	-5.7 to -0.5	**0.021[3]**

Characteristics	WLM3P		LCD		Difference	95% CI	*P* value
	n		n				
Δ Diastolic Blood Pressure (mm Hg), median(P25|P75)	46	-7.5 (-11.5| -4.0)	46	-6.0 (-9.0|-2.9)	-3.1	-5.7 to -0.5	**0.021**[3]
Δ HDL-C (mg/dL), mean (sd)	46	+7.9 (8.2)	45	+4.9 (6.2)	3.3	0.3 to 6.3	**0.029**[1]
♀ >50 mg/dL	27	81.8%	26	81.3%			0.079[2]
♀ <50 mg/dL	6	18.2%	6	18.8%			
♂ >40 mg/dL	11	84.6%	11	84.6%			0.412[2]
♂ <40 mg/dL	2	15.4%	2	15.4%			
LDL<115 (mg/dL)	23	50.0%	23	51.1%			0.916[2]
LDL≥115 (mg/dL)	23	50.0%	22	48.9%			
ΔLDL-C (mg/dL), median (P25|P75)	46	2 (-12.5 |13)	45	-4 (-16 |8)	5.8	15.1 to - 3.5	0.222[3]
ΔTG (mg/dL), median (P25|P75)	46	-26.5 (-50 to -9.8)	45	-25 (-64.5 to 7.0)	-8.7	-33.7 to 16.2	0.488[3]
<150	43	93.5%	42	93.3%			0.978[1]
>150	3	6.5%	3	6.7%			
ΔTG/HDL-C ratio median (P25-P75)	46	-1.2 (2.3)	45	-1.0 (1.2)	-0.4	-1.2 to 0.3	0.267[1]
♀ > 2.5	1	3%	4	12.5%			0.394[1]
♀ < 2.5	32	97%	28	87.5%			
♂ >3.5	2	15.4%	1	7.7%			0.246[1]
♂ <3.5	11	84.6%	12	92.3%			

[1]p values were computed with a linear regression model considering as confounders: age, sex, body mass index, and baseline glucose (used since differences were observed at baseline); [2]Chi-square test; [3]T-test for independent samples; WLM3P – Weight Loss Maintenance 3 Phases Program; LCD – low-carbohydrate diet; CI - confidence intervals; ♀ - female gender; ♂ - male gender; HDL-C - high-density lipoprotein cholesterol; TG – triglycerides

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At 6 months, there were no statistical differences between the groups according to SCORE2 (p=0.100) although in both groups there was a decrease in participants with high CVD risk compared to baseline. The low-to-moderate CVD risk was 89.1% (n=41) in WLM3P and 91.1% (n=41) in LCD; the high CVD risk was 10.9% (n=5) in WLM3P and 8.9% (n=9) in LCD group; and no participants with the category of very high CVD risk (Table 8).

Table 8. 6 months SCORE2 categories of study participants

	WLM3P **n=46**	**LCD** **n=45**	***P* value**
Low-to-moderate CVD risk, n (%)	41 (89.1%)	41 (91.1%)	0.100[1]
High CVD risk, n (%)	5 (10.9%)	4 (8.9%)	0.100[1]

[1]Chi-Square test; CVD- Cardiovascular Disease

Baseline energy consumption (kcal), carbohydrate intake (% of Total Energy Intake (% of TEI), protein intake (% of TEI), fat intake (% of TEI), saturated fat intake (% of TEI), and fiber intake (g/day) were not significantly different between the two groups (p>0.05).

At 6 months the TEI (kcal per day), carbohydrate intake (% of TEI), protein intake (% of TEI) and fiber intake (g/day) had statistically significant differences between groups (p<0.001). At 6 months the WLM3P had a lower TEI (kcal per day) compared to LCD [1313.8 (1167.5|1406.8 kcal vs 1444.4 (1304.6|1573.6) kcal; p<0.001]; a lower carbohydrate intake (% of TEI) compared to LCD [16.7 (14.4|24.6)% vs 25.1 (22.1|28.6)%; p<0.001], a higher protein intake (% of TEI) compared to LCD [31.8 (28.6|34.2)% vs 27.0 (24.6|29.4)%; p<0.001] and also a higher fiber intake (g/day) compared to LCD [19.2 (15.5|21.3)g vs 15.1 (12.9|18.8)g; p=0.040]. These results are described in Table 9.

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Table 9. Nutritional intake between WLM3P and LCD groups at baseline and 6 months

	Baseline			6 Month			
	WLM3P n=59	**LCD** n=53	***P* value[1]**	**WLM3P** n=46	**LCD** n=46	***β* (95% CI)**	***P* value[1]**
TEI (kcal per day) median (P25-P75)	2073.3 (1810.7|2308.1)	2064.5 (1876.2|2391.4)	0.437	1313.8 (1167.5|1406.8)	1444.4 (1304.6|1573.6)	-209.6 (-306.6 to -112.5)	**<0.001**
CHO (% of TEI) median (P25-P75)	39.6 (32.2|44.9)	39.3 (32.1|42.9)	0.308	16.7 (14.4|24.6)	25.1 (22.1|28.6)	-6.1 (-9.3 to -2.9)	**<0.001**
PROT (% of TEI) median (P25-P75)	18.6 (16.9|21.9)	19.7 (17.3|22.6)	0.327	31.8 (28.6|34.2)	27.0 (24.6|29.4)	5.9 (8.0 to 3.9)	**<0.001**
FAT (% of TEI) median (P25-P75)	38.1 (32.8|42)	38 (34.8|43.2)	0.461	45.6 (42.3|48.7)	44.4 (40.4|48.9)	0.6 (3.1 to -1.9)	0.661
SAT. FAT (% of TEI) median (P25-P75)	14.5 (12.6|17.3)	14.1 (11.7|16.0)	0.142	11.4 (10.5|13.0)	11.7 (10.2|13.2)	-0.8 (-1.8 to 0.3)	0.159
FIBER (g per day) median (P25-P75)	17.9 (15.0|23.9)	18.4 (14.9|22.5)	1.000	19.2 (15.5|21.3)	15.1 (12.9|18.8)	1.9 (4.7 to -0.9)	**0.040**

[1]T test for independent samples; WLM3P - Weight Loss Maintenance 3 Phases Program; LCD – low-carbohydrate diet; ***β*** – beta-coefficient; CI - confidence intervals; TEI - Total Energy Intake; CHO - Carbohydrate; PROT - Protein; SAT – Saturated

A positive correlation between TG (r=0.38; p=0.011) and protein intake at 6 months in the LCD and a positive correlation between TG (r=0.53; p<0.001) and fiber intake at 6 months in the WLM3P were found. No other correlations were observed.

At 6 months. the monthly therapeutic adherence based on self-reported was rated from 0 (none, 0%) to 5 (perfect, 100%) and classified as inferior or superior at 75% (2 categories). The WLM3P group has a therapeutic adherence ≥ 75% higher than the LCD group statistically significant (80.4% vs 58.7%; p=0.023). Table 10 describes these results.

Table 10. Therapeutic adherence at 6 months

Classification of therapeutic adherence			Group			
			WLM3P	**LCD**	Total	***P* value**[1]
			n=46	**n=46**		
	<75%	n (%)	9 (19.6%)	19 (41.3%)	28	**0.023**
	≥75%	n (%)	37 (80.4%)	27 (58.7%)	64	

[1]Chi-Square test; WLM3P - Weight Loss Maintenance 3 Phases Program; LCD – low-carbohydrate diet

Twenty participants dropped out, namely 22.0% (n=13) at WLM3P and 13.2% (n=7) at LCD (p=0.223) after 6 months.

Adverse side effects were moderate and transitory in both groups and were higher in the WLM3P (23.9% vs. 6.5%, p=0.020) at 6 months. At 1 month, constipation was the most frequent side effect reported in the intervention group (p=0.008). Table 11 describes the incidence of side effects in both groups.

Table 11. Incidence of side effects at 1 month and 6 months in both groups

Adverse effects,	**Month**	**Intervention Group**	**Control Group**	***P* value**[1]
***Yes,* n(%)**	1	20 (35.7)	11 (20.8)	0.084
	6	11 (23.9)	3 (6.5)	**0.020**
Constipation, n(%)	1	15 (26.8)	4 (7.5)	**0.008**
	6	5 (10.8)	2 (4.3)	0.238
Fatigue, n(%)	1	5 (8.9)	4(7.5)	0.793
	6	-	-	-
Headaches, n(%)	1	2 (3.6)	2 (3.8)	0.955
	6	-	-	-
Irritability, n(%)	1	0 (0)	2 (3.8)	0.142
	6	-	-	-
Diarrhea, n(%)	1	0 (0)	1 (1.9)	0.302
	6	-	-	-
Nauseas, n(%)	1	1 (1.7)	0 (0)	0.155
	6	-	-	-
Hair loss, n(%)	1	-	-	-
	6	4 (8.7)	1 (2.2)	0.168

[1]Chi-Square test

DISCUSSION

This study provides evidence on the effectiveness of WLM3P on lipid profile, an important concern in current options for obesity treatment[45]. After 6 months, our data showed that WLM3P increased HDL-C, decreased diastolic blood pressure and greater weight loss compared to LCD with a statistical difference. The cardiovascular risk according to TG/HDL-C ratio and SCORE2 was reduced in both groups but without statistical differences between them.

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The WLM3P was found to be more effective in increasing HDL-C compared to LCD and more effective compared to popular named diets for WL. The results of TG (median -26.5 mg/dL) and HDL-C (mean 7.9 mg/dL) of WLM3P indicate an improved lipid profile compared to the results of the recent meta-analysis and systematic reviews[39,50,54–57]. Dong, T. and colleagues[54] assess the relationship between LCD and cardiovascular risk factors. The LCD was associated with increased plasma HDL-c levels of 3.87 mg/dL[54]. Ge, L. and colleagues[39] determine the relative effectiveness of dietary macronutrient patterns and popular named diet programs for cardiovascular risk factor improvement in comparison with the usual diet. No popular named diets showed a statistically significant increase in HDL-C at the 6-month follow-up (the only ones with moderate to high certainty were Jenny Craig (median of 2.85 mg/dL) and Biggest Loser Slimming (median of 0.01 mg/dL))[39]. Nordmann, A.J. and colleagues[55] analysed the effects of low-carbohydrate vs low-fat diets on weight loss and lipid profile factors. This meta-analysis of randomized controlled trials showed an HDL-C, weighted mean difference, of 4.6 mg/dL (95% CI, 1.5-8.1 mg/dL)[55]. Furthermore, the results of HDL-C of WLM3P compared to other weight-loss programs analysed in a systematic review of Mehta, A.K. and colleagues[50], which are like Jenny Craig, Weight Watchers, Nutrisystem, Health Management Resources, Medifast, OPTIFAST, SlimFast, The Biggest Loser Club, indicates a higher improvement in this outcome. In some of these weight-loss programs, the HDL-C decreased and in others did not increase significantly, except in Jenny Craig in which in some trials the HDL-C increased by 9 mg/dL at 6 months. This result is inconsistent because, in some Jenny Craig trials, the HDL-C decreased (-12 mg/dL) and in others increased by just 1mg/dL[50]. The Network Meta-Analysis and Nutritional Geometry Approach of Liang S. and colleagues[57] analysed the relationship between macronutrient composition and non-communicable disease, including the biomarker HDL-C. The highest HDL-c levels were associated with diets comprised of 30% energy from protein, ≤40% from carbohydrates, and ≥35% from fat (similar to WLM3P). Additionally, the low carbohydrate high-fat diet significantly improved HDL-C when compared to the Dietary Approaches to Stop Hypertension diet, plant-based diet, low-fat diet, dietary guidelines-based diets, and Mediterranean diet (mean effect size (95% CI): 7.35, 7.35, 6.57, 6.19 and 4.64 mg/dL, respectively). The low carbohydrate high-fat diet was ranked the best at increasing HDL-C at 92.6%, according to the surface under the cumulative ranking curves (SUCRA) which indicates a greater chance of the treatment being the best for achieving this favourable outcome. Silverii, G.A. and colleagues[56] assess whether low-carbohydrate diets are associated with differences in lipid profile, compared to control non-carbohydrate–restricted diets. A reduction in TG was observed at 3-4, 10-14 and 18-30 months [median −1.78-20.63 (−35.37, −5.89), −27.09 (−38.29, −15.90) and −23.26 (−45.53, −0.98) mg/dl, respectively][56].

Although there were no statistical differences between groups at 6 months in the SCORE2 categories, both groups reduced the % participants with high CVD risk. According to scientific literature, this effect probably happened because of the diet-induced reduction of atherogenic lipoproteins and CVD risk[58]: Both dietary prescriptions involve substituting saturated fatty acids with unsaturated fatty acids. This is closer to the recommended dietary intake of less than 10% of TEI from saturated fatty acids. The prescriptions also emphasize the consumption of high-quality carbohydrates, protein-rich foods, and high-fiber foods. Together, these dietary changes can significantly reduce levels of LDL-C and TG, thereby reducing the risk of cardiovascular disease[59–62].

The difference in protein intake (%) of the WLM3P compared to the LCD [31.7 (± 4.4) vs 26.6 (± 5.7); p<0.001] may account for the improvement in the blood lipid profile enhanced in the WLM3P. The direct effects of increasing

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dietary protein on lipid metabolism are thought to be beneficial for plasma lipid profiles. First, dietary proteins are theoretically supposed to have hypocholesterolemic features by increasing hepatic bile acid synthesis[63]. Second, increases in hepatic β-oxidation and ketogenesis by dietary proteins have also been reported. The promotion of hepatic amino acid catabolism, which as an energy-requiring process increases hepatic lipid oxidation[64], and the stimulation of glucagon secretion, which promotes hepatic ketogenesis[65], was suggested as the underlying mechanism. Reduced production and accelerated clearance of chylomicrons through stimulation of lipoprotein lipase (LPL) also occur with increased intakes of proteins[66]. In addition, the hydrophobic nature of dietary proteins delays the digestion and absorption kinetics of triglyceride-rich chylomicrons[67]. Additionally, the decreased TG might be a result of lower de *novo lipogenesis* due to the lower carbohydrate content (%) [68–70] of the WLM3P compared with LCD [20.0 (± 8.4) vs (26.4 (± 6.7); p<0.001]. Decreasing carbohydrate intake from 20 to 50g per day initially and then gradually increasing it (120–150g/per day) is an intervention that has proven beneficial for TG levels[71], like in WLM3P. The metabolic effects of dietary fibers may be linked to the type of fiber and its fermentability[72], although the study did not evaluate these characteristics with the decrease of TG observed.

The dietary supplements of WLM3P may contribute to the lipid profile improvements shown through silymarin[73,74], green tea (catechins)[56,75–79], fructo-oligosaccharides[80], galacto-oligosaccharides[81,82], garcinia cambogia[83–85] and L-carnitine[86–91]. There is growing evidence supporting that silymarin supplementation holds significant cardiovascular protective properties, showing reduced levels of TG, LDL-C and increased HDL-C levels in pre-clinical studies[73,74]. Catechins, the major polyphenolic compounds in green tea, exert vascular protective effects through multiple mechanisms, including antioxidative, anti-hypertensive, anti-inflammatory, anti-proliferative, anti-thrombogenic, and lipid-lowering effects[75]. Meta-analyses have shown that green tea reduces total cholesterol and LDL-C but not HDL-C in both subjects with normal weight and overweight/obesity[56,76–79,92]. Several studies have shown that garcinia cambogia plays an important role in the regulation of endogenous lipid biosynthesis[83]. Triglycerides and LDL-C were significantly reduced in studies with the intervention of garcinia cambogia[84,85]. Some studies found that l-carnitine supplementation showed a significant effect on the reduction of TC, LDL-C and increased HDL-C[87,91] but no effect of l-carnitine was detected in TG[86]. Chromium and carnitine co-supplementation decreased triglycerides, total and LDL-C[88,89]. A meta-analysis revealed that L-carnitine supplementation significantly reduced TC, LDL-C and TG and also increased HDL-C[C87]. The risk of nutritional deficiencies is an important problem that may occur in a diet where a macronutrient is strictly avoided, while even with supplementation of essential micronutrients, the scientific community should not forget the synergistic effect of foods' micronutrients and antioxidants[93]. One of the WLM3P supplements is a vitamin and mineral supplement with fructo-oligosaccharides and galacto-oligosaccharides. Therefore fructo-oligosaccharides and galacto-oligosaccharides intake may have a beneficial effect on lipid metabolism and regulation of serum cholesterol levels in individuals that change their lifestyle. Fructo-oligosaccharides and galacto-oligosaccharides supplementation may be a strategy for lowering cholesterol, as shown in pilot studies and systematic reviews and meta-analysis of data from randomized controlled trials[80–82]. Multivitamins and mineral supplements might contribute to a significantly lower TC and LDL-C[94,95] and significantly higher HDL-C[94,96]. Despite the promising evidence described, more evidence and studies are needed to validate these effects on lipid profiles.

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Other WLM3P characteristics that might contribute to the results shown on HDL-C and/or TG are 14:10 time-restricted eating (14 hours of metabolic fasting and restriction of duration for eating to 10 hours)[97–102]. The 14:10 time-restricted eating of the WLM3P reinforces eating earlier in the day to be aligned with metabolic circadian rhythms. Proposed mechanisms for the increase of HDL-C and decrease of the lipoproteins directly related to LDL-C through time-restricted eating. Lipid profile improvement over TRE is caused by molecule modulation in the liver. Nuclear expression of peroxisome proliferator-activated receptor alpha and peroxisome proliferator-activated receptor-gamma coactivator primarily occurs, which leads to increased fatty acid oxidation and apolipoprotein A production, whereas apolipoprotein B decreases. The increased production of apolipoprotein A, a major component of HDL-C, contributes to the rise in HDL-C levels. Stimulated fatty acid oxidation leads to reduced hepatic triglycerides, decreased VLDL production, and lower serum levels of VLDL, LDL-C, and small dense low-density lipoprotein-cholesterol (sdLDL). As a result of decreased VLDL, LDL-C and sdLDL levels, there is a loss of transported cholesterol and TG within them, which is reflected in the reduction of serum cholesterol and TG through TRE[94].

Overall, the relationship between many dietary macronutrients and serum lipids, particularly apolipoproteins and lipid ratios, is not fully understood[103,104].

The dietary prescription of protein was 35 to 40% of TEI and the dietary intake at 6 months was 31,8% of TEI in the WLM3P. In the LCD, the dietary prescription of protein was 35 to 40% of TEI and the dietary intake at 6 months was 27% of TEI. Both groups had a lower fat dietary prescription than the fat dietary intake at 6 months. The WLM3P had a fat dietary prescription with 35 to 40% of TEI and a fat dietary intake at 6 months of 45.6% of TEI. The dietary prescription of fiber was higher than the dietary intake at 6 months in both groups, despite not having reached the recommended 20g per day. The dietary prescription of fiber was 20g to 25g per day in both groups and the dietary intake at 6 months was 19,2g per day in the WLM3P and the LCD was 15.1g per day.

The study has as strengths the clear definition of the target population for the dietary intervention (i.e. only subjects with obesity) that increases the reliability of results; trained and certified nutritionists collected all data and followed quality control protocols and there were high follow-up rates and adherence (22.0% dropped out after 6 months and 80.4% therapeutic adherence >75%), which, in medical care, better adherence is hypothesized to result in better treatment outcomes[105]. A consistent positive relationship between adherence and dropout to lifestyle modification programs and obesity outcomes was reported in previous studies[106–112]. Other studies for weight loss at 6 months of intervention indicate a number of dropouts of 22.6%[61], 23.9%[113], 27%[114], 44.4%[115] 47%[116] and 57%[117]. Furthermore, a different intervention to treat obesity, based on the administration of Liraglutide showed a dropout rate of 70.1% within 6 months[118]. The overall adherence rate for various weight loss interventions was 60.5%[119].

Another strength is the regular and frequent attendance of clinical visits including motivational support which is associated with weight loss outcomes[120,121].

Some limitations in this study should be addressed: the effect of confounding variables such as the large age range (18–65 years) of participants eligible to be included and an unbalanced gender representation (more women looking for weight loss programs)[122,123]. Another limitation is addressed to the food intake based on dietary recall despite the analysis of more than 1 food day (mean of 3 days in the study which is the most commonly used tool in food-based

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randomized controlled trials to assess and monitor intakes[124]). The measurement errors, such as under-or over-reporting of certain types of foods, and participants that may forget to report certain foods or report what is expected rather than their actual food intake[125] are the major limitations of dietary recall. Relying on memory may lead the study participant to omit or misreport consumed foods[126]. Difficulties with recalling quantities and frequencies of food intake have been well established in the literature[131–133]. Portion size estimation is a major concern mostly because is determined by perception, conceptualization and memory[134,135]. Mixed dishes are consumed during main meal occasions[138]. The proportions and quantities of the individual foods in mixed dishes vary by participant, which is more likely to be determined by individual consumer preference and food availability in the household, rather than physically measuring the actual quantities[139]. Moreover, the intake of foods and beverages from an individual tends to change from day to day. The fluctuations around an individual's usual mean intake reflect true eating habits under free-living conditions[140]. The education provided by the intervention arms in clinical trials may also influence dietary intake reporting[141,142]. Notwithstanding this, given the acknowledgement of the limitations of food-based randomized controlled trials, advances in the dietary intake data quality in clinical research settings may also provide insights into the dietary intake data derivation process of community-based intervention research and cohort studies[143].

CONCLUSION

Our data showed that the WLM3P, which combines a high-protein low-carbohydrate diet, weekly consultations with a nutritionist, use of food supplements, motivational support, 14:10 TRE, high-protein specific food and online platform monitoring, was found to be more effective in increasing HDL-C and decreasing weight compared to LCD after a 6-month intervention for WL. These results show that WLM3P can improve patient obesity care, enable evidence-based recommendations for WL, and improve lipid profile. More studies are needed to understand the effects of WLM3P in the long term.

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ATTACHMENTS

1. Approval by Ethics Committee

Decisão final sobre o projecto "Eficácia da Intervenção Nutricional M3F em Adultos com Obesidade"

A Comissão de Ética da NMS|FCM-UNL (CEFCM) decidiu, por unanimidade, aprovar o projecto de investigação intitulado "Eficácia da Intervenção Nutricional M3F em Adultos com Obesidade" (nº108/2018/CEFCM), submetido por Professora Doutora Maria Conceição Calhau.

Lisboa, 22 de Fevereiro de 2019

O Presidente da Comissão de Ética,

(Prof. Doutor Diogo Pais)

TO WHOM IT MAY CONCERN

The Ethics Research Committee NMS|FCM-UNL (CEFCM) has unanimously approved the Project entitled "Eficácia da Intervenção Nutricional M3F em Adultos com Obesidade" (nr.108/2018/CEFCM), submitted by Maria Conceição Calhau, MD, PhD.

Lisbon, February 22th, 2019

The Chairman of the Ethics Research Committee,

(Diogo Pais, MD, PhD)

Campo dos Mártires da Pátria, 130 | 1169-056 Lisboa | Portugal | T. +351 218 803 000 – Ext. 20447 E-mail cefcm@fcm.unl.pt

2. Informed consent

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INFORMAÇÃO AO PARTICIPANTE

Eficácia de Intervenções Nutricionais em Adultos com Obesidade

Investigadora responsável: Conceição Calhau, Professora Catedrática da NOVA Medical School | Faculdade de Ciências Médicas da Universidade NOVA de Lisboa e 0

Co-investigadores:
Marta P. Silvestre, Professora Auxiliar Convidada da NOVA Medical School | Faculdade de Ciências Médicas da Universidade NOVA de Lisboa e CINTESIS – Centro de Investigação em Tecnologias e Serviços de Saúde

André Rosário, Professor Auxiliar Convidado da NOVA Medical School | Faculdade de Ciências Médicas da Universidade NOVA de Lisboa e CINTESIS – Centro de Investigação em Tecnologias e Serviços de Saúde

Inês Mota, Técnica Superior da NOVA Medical School | Faculdade de Ciências Médicas da Universidade NOVA de Lisboa e CINTESIS – Centro de Investigação em Tecnologias e Serviços de Saúde

Está a ser convidado a participar num estudo de intervenção clínica com a duração de 18 meses cujo objetivo é avaliar a eficácia de programas de perda e manutenção do peso em indivíduos com excesso de peso (obesidade).

A sua participação no estudo é voluntária. Caso decida participar tem o direito de, a qualquer momento, desistir do estudo, sem prejuízo para o seu estado de saúde ou qualquer consequência.

Para aceitar participar deve ler a descrição do estudo até ao fim e assinar no espaço apropriado. Caso surja alguma dúvida na interpretação do texto ou questão relacionada com o estudo, esclareça junto do investigador, por favor.

Fundamentação e temática

A Organização Mundial de Saúde define a obesidade como uma acumulação excessiva de gordura, que promove alterações morfológicas e fisiopatológicas, responsáveis por reduzir o tempo e a qualidade de vida. Esta doença representa um dos maiores problemas de saúde pública e a sua prevalência tem vindo a aumentar a nível global e nacional nas últimas décadas. Em Portugal, 58,1% da população adulta apresenta excesso de peso (21,6% com obesidade e 36,5% pré-obesidade).

Os três principais objetivos de um programa para tratamento do excesso de peso são: diminuir o peso corporal, reduzir o risco de comorbilidades associadas, a longo prazo, e evitar o reganho de

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peso. A eficácia de um programa de emagrecimento depende de uma perda de peso clinicamente significativa, a qual é definida como uma perda intencional de pelo menos 5-10% do peso inicial e a sua manutenção por um período mínimo de 1 ano. Contudo, a maioria dos estudos de intervenção nutricional relatam apenas uma perda de peso média modesta, ou seja, <5% do peso inicial, após 12 meses.

Nos últimos anos, as dietas pobres em hidratos de carbono (dieta *low carb*) têm vindo a ser cada vez mais utilizadas num curto período de tempo, pelo facto de maximizarem a oxidação de gordura e conduzirem a um maior gasto energético. No entanto, a sua eficácia a longo prazo ainda não está comprovada. Por esse motivo, o debate sobre qual a melhor abordagem nutricional a utilizar mantém-se. Além disso, nos últimos anos tem-se estudado a relação entre o microbiota intestinal (bactérias que vivem no intestino de todos os indivíduos) e a obesidade. O microbiota intestinal é o novo desafio terapêutico no combate à obesidade. Um maior conhecimento sobre a influência do microbiota intestinal no peso corporal permitirá encontrar intervenções nutricionais específicas segundo as necessidades individuais.

Este ensaio clínico pretende avaliar a eficácia de programas de emagrecimento, que têm como principal objetivo a perda de peso, bem como o desenvolvimento de novos hábitos alimentares, mais saudáveis, que assegurem a manutenção do peso perdido no futuro. Estas metas são asseguradas mediante um acompanhamento regular, onde se pretende aplicar o programa, monitorizar os resultados, esclarecer dúvidas atempadamente e aconselhar nas escolhas alimentares.

Objetivos do estudo

Avaliar a efetividade de um programa de emagrecimento através de medições antropométricas e de composição corporal (ex: peso corporal, massa gorda, massa magra, gordura visceral, perímetro da cintura e da anca); avaliação da pressão arterial; análises ao sangue (ex: glicemia, insulina, marcadores inflamatórios, colesterol, triacilglicéridos); caracterização do microbiota intestinal (através da análise das fezes) e realização de questionários.

Quem pode participar neste estudo?

Serão selecionados voluntários que cumpram com os critérios de elegibilidade previamente definidos para o presente estudo, nomeadamente:

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Critérios de inclusão:

a) Idade superior a 18 e inferior a 65 anos de idade;

b) Índice de massa corporal ≥ 30 kg/m² e ≤ 39,9 kg/m²;

c) Aceitar participar num programa de emagrecimento;

d) Assinar o Termo de Consentimento Livre e Esclarecido.

Critérios de exclusão:

a) Estar a ser seguido por um clínico ou nutricionista com o objetivo de redução de peso, o que inclui a toma de medicação para perda de peso ou ter realizado cirurgia bariátrica;

b) Estar grávida e/ou a amamentar, nem estar a planear engravidar durante os próximos meses;

c) Ter insuficiência renal (insuficiência renal aguda ou crónica);

d) Ter incontinência urinária;

e) Ter cancro ou ter recebido tratamento há menos de 5 anos;

f) Ter doença do fígado grave (cirrose hepática ou hepatite crónica);

g) Ter doença inflamatória intestinal crónica (doença *Crohn*, colite ulcerosa e diverticulite);

h) Ter doença celíaca;

i) Ter insuficiência cardíaca grave (doença cardiovascular isquémica, angina, estável ou não estável; enfarte do miocárdio prévio, qualquer acidente vascular cerebral ou ser portador de pacemaker);

j) Ter doença autoimune (lúpus, artrite reumatoide);

k) Ter diabetes;

l) Ter doença do foro neurológico ou psiquiátrico (ex: doença de parkinson ou que esteja a tomar mais de 2 antidepressivos);

m) Ter alterações na coagulação sanguínea (hemofilia; controlo regular do INR e toma de anticoagulantes orais);

n) Ter doenças do comportamento alimentar (ex: anorexia e bulimia);

o) Seguir regime alimentar vegetariano ou vegan;

p) Ter alergias/intolerâncias severas a alimentos ou medicamentos.

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Apresentação do estudo

O estudo será organizado em **várias sessões de acompanhamento regular** (no mínimo 1 vez por mês e no máximo 1 vez por semana) **a definir por um Nutricionista, ao longo dos 18 meses do estudo.**

Note-se que nas deslocações à NMS|FCM, as intervenções estão organizadas para que disponha de aproximadamente **2 horas do seu tempo**. O grupo de investigação não se responsabiliza pelos gastos associados à deslocação. Não está preconizado qualquer pagamento ou compensação pela sua participação.

O estudo irá ocorrer no Centro de Estudos de Doenças Crónicas (CEDOC) da NOVA Medical School|Faculdade de Ciências Médicas (NMS|FCM) da Universidade NOVA de Lisboa, que se situa na Rua Câmara Pestana 6, 1150-082 Lisboa.

Este estudo teve a aprovação da Comissão de Ética da NOVA Medical School|Faculdade de Ciências Médicas da Universidade NOVA de Lisboa, com o número de registo 108/2018/CEFCM.

O protocolo deste estudo clínico foi formalmente registado, e pode ser consultado, numa base de dados pública de registo – ClinicalTrials.gov (https://clinicaltrials.gov/), com o número de registo NCT04192357.

Os resultados do estudo clínico serão publicados numa revista científica com revisão por pares, independentemente dos resultados do estudo (http://www.ncbi.nlm.nih.gov/pubmed)..

Protocolo

1. Recrutamento e seleção (1ª visita):

Para avaliar a sua elegibilidade para ser incluído no estudo, será chamado para um rastreio inicial no gabinete clínico do CEDOC, NOVA Medical School|Faculdade de Ciências Médicas da Universidade NOVA de Lisboa (NMS|FCM).

A fim de determinar a sua elegibilidade, realizar-se-á uma entrevista clínica na qual serão realizadas as seguintes avaliações:

a) Medições antropométricas: peso (kg), estatura (m) e índice de massa corporal (kg/m²);
b) Questionário geral: estado de saúde e medicação.

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Caso satisfaça os critérios de elegibilidade estabelecidos para este estudo, ser-lhe-á explicado em detalhe todos os procedimentos envolvidos e entregue o seguinte material: 1) a informação detalhada sobre o **protocolo do estudo**, 2) o **consentimento informado**, 3) o ***kit* de recolha de fezes (análise das bactérias intestinais)** e 4) o **registo alimentar** com o intuito de se recolher informações sobre hábitos alimentares.

Se for elegível, será contactado para agendamento da segunda visita (*baseline)* à NMS|FCM.

Se não for elegível, os dados que forneceu durante o rastreio serão destruídos e não voltaremos a contactá-lo no âmbito deste estudo.

2. *Baseline* (2ª Visita, apenas para participantes elegíveis):

Os participantes elegíveis serão contactados para agendamento da 2ª visita à NMS|FCM para iniciar o estudo.

No dia da visita deverá chegar à NMS/FCM à hora agendada e cumprindo estes pré-requisitos:

- Fazer um **jejum** de 10 a 12 horas, no máximo de 15 horas de alimentos líquidos e sólidos;
- Pode beber água até 4 horas antes da visita;
- Não realizar exercício físico nas 24 horas anteriores ao dia da visita;
- Não ingerir bebidas ricas em cafeína (chá preto, café, refrigerantes com cafeína), alcoólicas ou drenantes nas 24 horas que antecedem a visita;
- Trazer a **recolha de fezes** utilizando o kit que lhe foi entregue para o efeito e seguindo as suas instruções de utilização. O kit deverá ser mantido à temperatura ambiente antes da sua utilização. Após utilização, guarde o kit preferencialmente no frigorífico até ao dia da visita. No dia da visita, pode trazê-lo à temperatura ambiente.
- Trazer o **registo alimentar** de 3 dias preenchido de acordo com as recomendações fornecidas.

Durante a visita serão realizadas as seguintes avaliações:

a) **Medições antropométricas** (peso (kg), perímetros da cintura e da anca (cm)) e avaliada a **composição corporal** através do método de bioimpedância elétrica. A bioimpedância trata-se de um método indolor, não-invasivo, mas cuja avaliação implica ficar de roupa interior e com uma bata hospitalar fornecida pela equipa de investigação. Este método fornece os dados da sua composição corporal, nomeadamente massa corporal total (peso), massa magra, massa gorda e percentagem de água corporal.

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Para a realização da bioimpedância elétrica, ser-lhe-á pedido que respeite os seguintes requisitos (para além dos descritos anteriormente):

- Utilizar a casa de banho antes da medição. As fezes e a urina irão contribuir para o peso total, pelo que é importante eliminar estes confundidores antes da medição;
- No caso das mulheres, o teste deverá ser feito fora do período de menstruação.

b) **Colheita de sangue** (as amostras recolhidas serão usadas para analisar os marcadores bioquímicos em estudo (glicose, insulina, marcadores inflamatórios, colesterol, triacilglicéridos, Vitamina D, marcadores da função renal e hepática).

c) **Medição da pressão arterial**.

d) **Questionários** (sociodemográfico; estado de saúde e medicação; crononutrição; atividade física; funcionamento intestinal).

No final será agendada a visita seguinte, aproximadamente, após 4 semanas.

3. Visita após 4 semanas (3ª Visita):

Além das visitas agendadas regularmente com o seu Nutricionista, após 4 semanas deverá chegar à NMS/FCM à hora agendada e cumprindo estes pré-requisitos:

- Fazer um **jejum** de 10 a 12 horas, no máximo de 15 horas de alimentos líquidos e sólidos;
- Pode beber água até 4 horas antes da visita;
- Não realizar exercício físico nas 24 horas anteriores ao dia da visita;
- Não ingerir bebidas ricas em cafeína (chá preto, café, refrigerantes com cafeína), alcoólicas ou drenantes nas 24 horas que antecedem a visita;
- Trazer o **registo alimentar** preenchido de acordo com as recomendações fornecidas.

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Durante a visita serão realizadas as seguintes avaliações:

e) **Medições antropométricas** (peso (kg), perímetros da cintura e da anca (cm)) e avaliada a **composição corporal** através do método de bioimpedância elétrica. A bioimpedância trata-se de um método indolor, não-invasivo, mas cuja avaliação implica ficar de roupa interior e com uma bata hospitalar fornecida pela equipa de investigação. Este método fornece os dados da sua composição corporal, nomeadamente massa corporal total (peso), massa magra, massa gorda e percentagem de água corporal.

Para a realização da bioimpedância elétrica, ser-lhe-á pedido que respeite os seguintes requisitos (para além dos descritos anteriormente):

- Utilizar a casa de banho antes da medição. As fezes e a urina irão contribuir para o peso total, pelo que é importante eliminar estes confundidores antes da medição;
- No caso das mulheres, o teste deverá ser feito fora do período de menstruação.

f) **Colheita de sangue** (as amostras recolhidas serão usadas para analisar os marcadores bioquímicos em estudo (glicose, insulina, marcadores inflamatórios, colesterol, triacilglicéridos, Vitamina D, marcadores da função renal e hepática).

g) **Medição da pressão arterial**.

h) **Questionários** (alteração da medicação; adesão ao protocolo; atividade física; alteração da crononutrição; funcionamento intestinal; satisfação com a intervenção nutricional; acontecimentos adversos).

No final será agendada a visita seguinte, aproximadamente, após 6 meses.

4. Visita aos 6 meses (4ª Visita):

Além das visitas agendadas regularmente com o seu Nutricionista, após 6 meses deverá chegar à NMS/FCM à hora agendada e cumprindo estes pré-requisitos:

- Fazer um **jejum** de 10 a 12 horas, no máximo de 15 horas de alimentos líquidos e sólidos;
- Pode beber água até 4 horas antes da visita;
- Não realizar exercício físico nas 24 horas anteriores ao dia da visita;
- Não ingerir bebidas ricas em cafeína (chá preto, café, refrigerantes com cafeína), alcoólicas ou drenantes nas 24 horas que antecedem a visita;

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- Trazer a **recolha de fezes** utilizando o kit que lhe foi entregue para o efeito e seguindo as suas instruções de utilização. O kit deverá ser mantido à temperatura ambiente antes da sua utilização. Após utilização, guarde o kit preferencialmente no frigorífico até ao dia da visita. No dia da visita, pode trazê-lo à temperatura ambiente.

- Trazer o **registo alimentar** preenchido de acordo com as recomendações fornecidas.

Durante a visita serão realizadas as seguintes avaliações:

i) **Medições antropométricas** (peso (kg), perímetros da cintura e da anca (cm)) e avaliada a **composição corporal** através do método de bioimpedância elétrica. A bioimpedância trata-se de um método indolor, não-invasivo, mas cuja avaliação implica ficar de roupa interior e com uma bata hospitalar fornecida pela equipa de investigação. Este método fornece os dados da sua composição corporal, nomeadamente massa corporal total (peso), massa magra, massa gorda e percentagem de água corporal.

Para a realização da bioimpedância elétrica, ser-lhe-á pedido que respeite os seguintes requisitos (para além dos descritos anteriormente):

- Utilizar a casa de banho antes da medição. As fezes e a urina irão contribuir para o peso total, pelo que é importante eliminar estes confundidores antes da medição;
- No caso das mulheres, o teste deverá ser feito fora do período de menstruação.

j) **Colheita de sangue** (as amostras recolhidas serão usadas para analisar os marcadores bioquímicos em estudo (glicose, insulina, marcadores inflamatórios, colesterol, triacilglicéridos, Vitamina D, marcadores da função renal e hepática).

k) **Medição da pressão arterial**.

l) **Questionários** (alteração da medicação; adesão ao protocolo; atividade física; alteração da crononutrição; funcionamento intestinal; satisfação com a intervenção nutricional; acontecimentos adversos).

No final será agendada a visita seguinte, aproximadamente, após 18 meses.

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5. Aos 18 meses (5ª Visita):

Aos 18 meses será agendada a última visita à NMS/FCM. Deverá chegar à NMS/FCM à hora agendada e cumprindo estes pré-requisitos:

- Fazer um **jejum** de 10 a 12 horas, no máximo de 15 horas de alimentos líquidos e sólidos;
- Pode beber água até 4 horas antes da visita;
- Não realizar exercício físico nas 24 horas anteriores ao dia da visita;
- Não ingerir bebidas ricas em cafeína (chá preto, café, refrigerantes com cafeína), alcoólicas ou drenantes nas 24 horas que antecedem a visita;
- Trazer a **última recolha de fezes** utilizando o kit que lhe foi entregue para o efeito e seguindo as suas instruções de utilização. O kit deverá ser mantido à temperatura ambiente antes da sua utilização. Após utilização, guarde o kit preferencialmente no frigorífico até ao dia da visita. No dia da visita, pode trazê-lo à temperatura ambiente.
- Trazer o **último registo alimentar** preenchido de acordo com as recomendações fornecidas.

Durante a visita serão realizadas as seguintes avaliações:

m) **Medições antropométricas** (peso (kg), perímetros da cintura e da anca (cm)) e avaliada a **composição corporal** através do método de bioimpedância elétrica. A bioimpedância trata-se de um método indolor, não-invasivo, mas cuja avaliação implica ficar de roupa interior e com uma bata hospitalar fornecida pela equipa de investigação. Este método fornece os dados da sua composição corporal, nomeadamente massa corporal total (peso), massa magra, massa gorda e percentagem de água corporal.

Para a realização da bioimpedância elétrica, ser-lhe-á pedido que respeite os seguintes requisitos (para além dos descritos anteriormente):

- Utilizar a casa de banho antes da medição. As fezes e a urina irão contribuir para o peso total, pelo que é importante eliminar estes confundidores antes da medição;
- No caso das mulheres, o teste deverá ser feito fora do período de menstruação.

n) **Colheita de sangue** (as amostras recolhidas serão usadas para analisar os marcadores bioquímicos em estudo (glicose, insulina, marcadores inflamatórios, colesterol, triacilglicéridos, Vitamina D, marcadores da função renal e hepática).

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o) **Medição da pressão arterial.**

p) **Questionários** (alteração da medicação; adesão ao protocolo; atividade física; alteração da crononutrição; funcionamento intestinal; satisfação com a intervenção nutricional; acontecimentos adversos).

Gostaríamos também de relembrar que haverá mais consultas intermédias além das visitas supracitadas (no mínimo 1 vez por mês e no máximo 1 vez por semana), as quais serão agendadas pelo seu nutricionista, ao longo dos 18 meses.

Benefícios previsíveis e eventuais riscos para os participantes

A sustentabilidade da perda de peso é uma preocupação crescente por parte dos profissionais de saúde e dos participantes que iniciam um programa de emagrecimento, o que determina a necessidade de se desenvolverem programas estruturados, que envolvam duas fases: perda e manutenção do peso. Uma redução de peso a longo prazo traduzir-se-á na melhoria da qualidade de vida, na diminuição da mortalidade e na melhoria das doenças crónicas associadas.

Desistências do estudo

Se desejar abandonar o estudo pode fazê-lo em qualquer momento, sem necessidade de apresentar justificação, ou sem qualquer prejuízo na assistência que é prestada. Tem também o direito de ver eliminados/apagados/destruídos todos os seus dados.

Propriedade e proteção de dados dos participantes/confidencialidade

Toda e qualquer informação pessoal (incluindo dados pessoais e de saúde inscritos nos questionários; resultados laboratoriais da análise do sangue e fezes recolhidos) será confidencial, estando sempre assegurada a sua privacidade.

Para esse efeito, as suas informações serão codificadas e tratadas de forma agregada, salvaguardando a sua confidencialidade. A recolha, codificação, processamento, armazenamento e destruição dos dados dos participantes é da responsabilidade da equipa de investigação.

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Especificações da recolha e destino final das amostras biológicas

As colheitas de sangue serão efetuadas por um(a) flebotomista/enfermeiro(a) treinado(a) para minimizar o desconforto inerente à recolha. O sangue e fezes recolhidos serão devidamente armazenados nos laboratórios da NMS|FCM até ao fim da análise das amostras e serão destruídas durante esse processo.

As colheitas de fezes serão utilizadas para a caracterização do microbiota intestinal.

As análises bioquímicas e às fezes serão realizadas por um laboratório de análises devidamente certificado, e coordenadas pela equipa de investigação (que será responsável pela codificação das amostras). Todos os dados contidos nas amostras biológicas serão tratados de forma agregada, salvaguardando a confidencialidade dos participantes.

Contactos

Se tiver alguma questão ou dúvida durante o estudo deverá contactar a equipa de investigação (email: pmp@nms.unl.pt , número de telemóvel: 93 249 41 64).

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APPENDIX B – INFORMED CONSENT FORM

CONSENTIMENTO INFORMADO, ESCLARECIDO E LIVRE

Considerando a "Declaração de Helsínquia" da Associação Médica Mundial
(Helsínquia 1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996; Edimburgo 2000; Seoul 2008; Fortaleza 2013)

Eu, abaixo-assinado, (nome completo) _____

_____

declaro que compreendi a explicação que me foi fornecida acerca da investigação no âmbito do projeto Eficácia de Intervenções Nutricionais em Adultos com Obesidade. Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsínquia, a informação ou explicação que me foi prestada versou os objetivos, os métodos, os benefícios previstos, os potenciais riscos e o eventual desconforto. Foi-me ainda dada a oportunidade de fazer as perguntas que julguei necessárias e, de todas, obtive resposta satisfatória.

Desta forma, declaro que:

- Permito a utilização dos dados que de forma voluntaria forneço, unicamente para esta investigação, sendo garantido o anonimato e confidencialidade;
- Compreendi que a minha participação no estudo é voluntária;
- Fui informado que a minha participação neste estudo não me confere o direito a qualquer tipo de remuneração;
- Fui informado que o grupo de investigação não se responsabiliza pelos gastos associados à deslocação.
- Compreendi os potenciais riscos e benefícios envolvidos na minha participação neste estudo;
- Fui informado que terei um tempo definido (1 semana) para considerar a minha participação neste estudo;
- Tenho conhecimento de quem devo contactar se tiver algum efeito secundário resultante deste estudo;
- Tenho conhecimento de quem devo contactar se tiver alguma dúvida sobre este estudo;
- Compreendi que posso desistir quando desejar sem apresentar qualquer justificação, não sendo de forma alguma comprometidos os futuros cuidados que receberei dos profissionais de saúde.

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A preencher pelos participantes (assinale a opção apropriada, por favor)		
Compreendi a explicação que me foi facultada acerca do estudo que se tenciona realizar, nomeadamente os objetivos, os métodos, os benefícios previstos, os potenciais riscos e o eventual desconforto; conforme informação ao participante em anexo.	Sim	Não
Aceito participar no estudo clínico - Eficácia de Intervenções Nutricionais em Adultos com Obesidade - com a duração de 18 meses.	Sim	Não
Aceito responder a questionários relativos a dados sociodemográficos e práticas de estilo de vida.	Sim	Não
Compreendi que posso ter acesso aos valores bioquímicos obtidos pela colheita de sangue, caso o pretenda e expresse via email.	Sim	Não
Aceito preencher 4 registos alimentares por forma a avaliar os meus hábitos alimentares	Sim	Não
Autorizo a realização de avaliações antropométricas (altura, peso, perímetro da cintura e anca) e a medição da pressão arterial.	Sim	Não
Aceito realizar avaliações da composição corporal através de bioimpedância elétrica, usando uma bata hospitalar por cima da roupa interior.	Sim	Não
Autorizo a recolha e análise ao sangue a fim de analisar a glicemia, marcadores inflamatórios, o perfil lipídico, Vitamina D, marcadores da função renal e hepática. A recolha das amostras de sangue será realizada por um(a) flebotomista/enfermeiro(a) treinado(a) para minimizar o desconforto.	Sim	Não
Autorizo fornecer uma amostra de fezes a fim de analisar as bactérias intestinais (microbiota intestinal).	Sim	Não
Autorizo que as minhas amostras de sangue e fezes recolhidas sejam devidamente armazenadas nos laboratórios da NOVA Medical School|Faculdade de Ciências Médicas da Universidade NOVA de Lisboa (NMS|FCM) e serão destruídas após o estudo, se eu for elegível para este estudo.	Sim	Não
Caso os resultados da análise ao sangue indiquem um potencial ou real risco para a minha saúde, gostaria de ser informado e entendo que o meu médico também seja notificado. Para além disto, concordo em não ser incluído do estudo caso os resultados da análise não sejam compatíveis com a minha participação.	Sim	Não
Autorizo a transmissão dos meus dados pessoais, nomeadamente o meu nome, contacto de telemóvel e email, entre os membros da equipa de investigação, para que possam entrar em contacto direto comigo durante o estudo, confiando em que apenas serão utilizados para esta investigação e na garantia de confidencialidade que me é dada pelo investigador e nos termos	Sim	Não

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da política de proteção de dados pessoais para este estudo, que me foi explicada pelo investigador.		
Autorizo que a equipa de investigação da NMS|FCM entre em contacto comigo se existirem estudos futuros para os quais seja elegível.	Sim	Não

Este documento é feito em duplicado (uma via para quem consente e uma via para o investigador).

Confirmo que expliquei ao participante, de forma adequada e compreensível, a investigação referida, os benefícios, os riscos e possíveis complicações associadas à sua realização.

Conceição Calhau _____

Assinatura da investigadora responsável

Declaro que forneci toda a informação detalhada relativa ao estudo infra descrito e que esclareci de forma clara e objetiva todas as dúvidas colocadas pelo voluntário.

Data: ___ / ___ /___ _____

Assinatura do Nutricionista investigador

Declaro ter lido e compreendido este documento, bem como as informações verbais que me foram fornecidas pelos investigadores deste estudo. Foi-me garantida a possibilidade de, em qualquer altura, recusar participar neste estudo sem qualquer tipo de consequências. Desta forma, aceito participar neste estudo e permito a utilização dos dados que de forma voluntária forneço, confiando em que apenas serão utilizados para esta investigação e nas garantias de confidencialidade e anonimato que me são dadas pelo investigador.

Data: ___ / ___ /___ _____

Assinatura do participante