Nutritional habits and attitudes in fitness center users

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Thank you to my mentor Assoc. Prof. Joško Božić, MD, PhD for the patience and great support during the last months.

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Thank you to my friends and of course my girlfriend for making the past six years an incredible journey which I will miss dearly as I start my professional career.

"Sometimes I'll start a sentence and I don't even know where it's going. I just hope I find it along the way."

LIST OF ABBREVIATIONS

MD – Mediterranean diet

NCD – non-communicable diseases

CVD – cardiovascular disease

WHO – world health organization

CDD – chronic degenerative disease

HTN – hypertension

HDL – high-density lipoprotein

LDL – low-density lipoprotein

DASH – dietary approaches to stop hypertension

PUFA – polyunsaturated fatty acids

RCT – randomized controlled trial

ROS – reactive oxygen species

TFA – transfatty acids

MUFA – monounsaturated fatty acids

SFA – saturated fatty acids

NAFLD – non-alcoholic fatty liver disease

AA – amino acids

ENS – enteric nervous system

CNS – central nervous system

NADH – nicotinamide adenine dinucleotide

NADPH – nicotinamide adenine dinucleotide phosphate

VCAM-1 – vascular cell adhesion molecule 1

VLA-4 – very late antigen 4

MCP-1 – monocyte chemoattractant protein 1

TGF-β – transforming growth factor

SMC – smooth muscle cells

PDGF – platelet derived growth factor

ECM – extracellular matrix

VSMC – vascular smooth muscle cell

MDSS – the Mediterranean dietary serving score

1. INTRODUCTION

1.1 Nutrition, its components and impact on cardiovascular disease

Nutrition is a large topic that has been thoroughly researched through many decades and continues to do so. Identifying beneficial and harmful nutrients stands to help decrease risk for non-communicable diseases (NCD) and can ultimately help to reduce the great burden these diseases have today (1-3). Diet is one of the modifiable risk factors including but not limited to tobacco, excessive alcohol consumption and physical inactivity for NCDs (2, 4). The world health organization (WHO) has created approaches to manage the aforementioned modifiable habits through the Global Action Plan for the Prevention and Control of NCD (4).

At the base of all healthy diets, you will find a combination of macro- and micronutrients, bioactive compounds and proper hydration without overfeeding, i.e. exceeding daily caloric expenditure (Figure 1) (3). The principal components of macronutrients are carbohydrates, proteins and fats. As the main source of energy, they are vital for the physiologic functioning of our body.

Figure 1. An overview of what a healthy lifestyle and diet contains. Source: Cena H, Calder PC. Defining a Healthy Diet: Evidence for The Role of Contemporary Dietary Patterns in Health and Disease. Nutrients. 2020;12:334.

1.1.1 Macronutrients

1.1.1.1 Carbohydrates

The bulk of dietary carbohydrates derives from whole grain, legumes, fruits and vegetables. They can be sorted into monosaccharides (glucose, fructose and galactose), the basic form from which all other carbohydrates are built namely, disaccharides (lactose, maltose and sucrose), oligosaccharides and polysaccharides. Glucose is the main fuel for ATP production in mitochondria. This how most of the cells in the human body produce energy. Fructose is the main monosaccharide in fruit. It has a lower impact on blood glucose level compared to glucose and is primarily metabolized in the liver. Lactose found in milk is cleaved by intestinal lactase and absorbed as glucose and galactose. Sucrose found naturally in plants is more commonly known as white sugar found in most households. Oligo- and polysaccharides unable to be absorbed in the intestinal tract are collectively known as fibers and has been related to a wide variety of health benefits (5).

1.1.1.2 Proteins

Most protein dense foods are animal derived, especially red meat, fish, chicken meat and eggs. Plants can also be good sources for protein particularly nuts and legumes. Proteins are chains of amino acids(AA) and play a vital role in the many tasks a cell performs. Examples of such tasks include catalyzing reactions as enzymes, DNA replication, cell signaling, transport, structure and building to name a few. There are 21 amino acids common to us and out of those, 9 are termed essential amino acids. The later cannot be synthesized de novo or is produced, but in insufficient amount relative to the demand. This means they must be incorporated through the diet. Additionally, 6 AAs are called conditionally essential due to their potential of becoming insufficient in certain pathologic states (5).

1.1.1.3 Fats

Out of the three main macronutrients fat contains the most energy, 9kcal/g or 37kJ while both carbohydrates and protein provide 4 kcal/g (17kJ) each. The nutrient consists of fatty acids, and we divide them into monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA) and trans fatty acids (TFA), based on the presence or absence of one or more double bonds between the carbon atoms (5). Foods rich in MUFA includes sunflower- and olive oil, avocado, peanuts and various nuts (almond, hazelnuts, pistachio). PUFA are found in some of the same types of food as MUFA like nuts (walnut, pine nut) but also abundant in fatty fish (salmon, mackerel and tuna), sunflower- and flax seeds. Saturated fat as its name suggests, only contains single bonds between the fatty acids. Rendering the carbon atoms free as there are no available spots for other atoms to bind. SFA are typically from animals (meat and dairy), processed and deep-fried food, coconut and palm oil(5). These three are the naturally occurring fats, this usually does not apply to TFA, as most is produced by an industrial process. It is an unsaturated fat with a double bond in the trans configuration (6). Studies indicate that TFA contributes to increased risk of CVDs by disturbing several biological pathways and promoting formation of atherosclerosis (7).

The aforementioned fatty acids have been extensively studied through the past decades. For many years the consensus has been that a diet with a large proportion of SFA and TFA relative to unsaturated fatty acids, like the western diet, is associated with an increased risk of CVDs. However, recent meta-analyses have put that claim under scrutiny, as there was no association between risk of CVD and intake of MUFA, PUFA and SFA except for TFA which did increase the risk (8).

1.1.2 Micronutrients

Micronutrients differ from macronutrients in the amounts required. Macronutrients may be your main source for energy, but micronutrients are equally as vital. They represent vitamins and minerals and are found in a wide variety of food. They play important roles for your immune system, hemostasis, growth and an insufficient intake can lead to a wide array of health conditions (9-12). Micronutrient deficiencies (MNDs) contributes to the major burden of disease in both low- and high-income countries (9).

The population at most risk for deficiencies are children less than 5 years of age and pregnant women (9). Folic acid supplementation is recommended for women both planning to get pregnant and during pregnancy to significantly reduce the risk for neural tube defects (10). Increased intake of iron is important to decrease the risk of iron deficiency anemia during menstruation or in periods of raised requirements such as pregnancy and infancy (9). Apart from its well-known role in bone hemostasis, vitamin D also plays a key role in your immune system. Deficiencies may lead to increased autoimmunity and susceptibility to infections (12). Another vitamin important for the immune system is vitamin A and insufficiency can lead to blindness (9).

Reaching the minimal requirements are therefore incredibly important. Supplementation of various minerals and vitamins is a common practice and has been researched for its potential health benefits. A meta-analysis from 2018 saw little to no effect from moderate- and low-quality evidence of vitamin and mineral supplementation to prevent CVDs. Supplementation of antioxidants and niacin was associated with an increased risk for all cause mortality rather than decreased risk for CVDs. B vitamins and folic acid was the only supplementation of documented benefit with a reduced incidence of stroke (13).

1.1.3 Cardiovascular disease and nutrition

Alongside cancer, CVD stands as the one of the top leading causes of death today (1). The Framingham study was one of the first major studies that identified risk factors related to CVD. It started in 1948 and is still an ongoing study. Major identified modifiable risk factors are tobacco use, hypertension, hyperlipidemia, diabetes, obesity and sedentary lifestyle with a low amount of physical exercise (14). Adopting a healthy lifestyle with physical activity, refraining from tobacco smoking and addressing conditions such as HTN and diabetes with appropriate medications are important initial steps in reducing risk of CVD (15). Appropriate nutrition also plays a big role as a modifiable risk factor (1, 3, 16).

When comparing a typical western diet consisting of processed food containing high amounts of fat, sugar and sodium with a more varied diet made up of higher amounts of fruit and vegetables such as the Mediterranean and Dietary Approaches to Stop Hypertension (DASH) diets. There is an increased activity of pro inflammatory cytokines in the former (1, 17). This further promotes formation of atherosclerosis as inflammation is the initial step in production of atherosclerosis (18). It would be reasonable to say that a diet with relatively lower in proinflammatory activity compared to other diets could help prevent CVD.

1.2 The Mediterranean diet

The Mediterranean diet (MD), as its name suggests, is a product of cultural dining habits originating from countries surrounding the Mediterranean Sea. It involves the raw ingredients available in the region, how the food is prepared, processed and consumed (2). It has become a well-known diet especially in regards to its role in preventing CVDs (19). Other health benefits have also been investigated with promising results, e.g. metabolic syndrome, cancer, brain diseases (19-24).

In 2008, a study was performed on a Greek population adhering to the MD in varying degree. The results revealed a 12% reduction in cancer incidence to those adhering to the diet, especially in women (20). Another meta-analysis from 2008 demonstrated a decreased mortality of CVDs and cancer by 9% and 6% respectively. Also seen in the study was a lower incidence of Alzheimer's disease and Parkinson 13% (21). More recent studies further support the inverse correlation between the MD and CVD´s (53).

At the diets core you'll find a large quantity of plant-based foods such as fruits, vegetables, nuts, olive oil, legumes and cereals. It also consists of a high intake of fish with moderate intake of red meat, poultry, eggs, dairy and sweets. Alongside meals wine, commonly red, is consumed but in moderate amounts (2). The benefits of the diet arrive from its high content of nutrients, namely: antioxidants, monounsaturated fats, fiber, omega-3 fatty acids, phytosterols, probiotics (3, 25, 26). The recommended food types and quantity can be depicted in diet pyramids (Table 1).

Table 1. Three Mediterranean diet pyramids, Oldway's Preservation and Trust (2009) Mediterranean Diet Foundation (2011) 1999 Greek Dietary Guidelines (1999)

Foods	**Oldway's Preservation and Trust, 2009**	**Mediterranean Diet Foundation, 2011**	**1999 Greek Dietary Guidelines, 1999**
Olive oil	Every meal	Every meal	Main added lipid
Vegetables	Every meal	≥2 serves every meal	6 serves daily
Fruits	Every meal	1-2 serves every meal	3 serves daily
Bread and cereal	Every meal	1-2 every meal	8 serves daily
Legumes	Every meal	≥2 Serves weekly	3-4 serves weekly
Nuts	Every meal	1-2 serves daily	3-4 serves weekly
Fish/Seafood	Often, at least two times per week	≥2 serves weekly	5-6 serves weekly
Eggs	Moderate portions, daily to weekly	2-4 serves daily	3 serves weekly
Poultry	Moderate portions, daily to weekly	2 serves weekly	4 serves weekly
Dairy foods	Moderate portions, daily to weekly	2 serves daily	2 serves daily
Red meat	Less often	< serves/week	4 servings monthly
Sweets	Less often	<serves week	3 servings weekly
Red wine	In moderation	In moderation and respecting social beliefs	Daily in moderation

Source: Davis C, Bryan J, Hodgson J, Murphy K. Definition of the Mediterranean Diet; a Literature Review. Nutrients. 2015;7:9139-9153.

1.2.1 Omega-3 fatty acids

Fish is one of the main sources for omega-3 fatty acids. Today in the western diet there's a disproportionate amount of omega-6 compared to omega-3 intake. Historically it is believed that the ratio of ω-6/ ω-3 was closer to 1:1 but today has approached 10:1, and 15-20:1 in the western diet (27). This increased ratio can lead to a proinflammatory state which promotes formation of atherosclerosis, while also contributing to autoimmune diseases and cancer (18, 28). Conversely the opposite may be observed when the ratio approaches 1:1 (28). Although several RCTs have demonstrated benefit of omega 3 in regard to CVD, the largest meta-analysis conducted on omega-3 fatty acids in relation to cardiovascular disease proved little to no effect of increasing dietary intake ω-3. To conclude the matter, despite reported benefits of omega-3 there is uncertainty to its true health benefits. Fish is however nutrient dense food rich in vitamins, proteins and minerals which is beneficial regardless of the impact on CVD (29).

1.2.2 Polyphenols and olive oil

Polyphenols are found in red wine, olive oil as well as other fruits and vegetables. It has been related to several health benefits through its antioxidant effect, reducing reactive oxygen species (ROS) (30). For low-density lipoprotein (LDL) to promote formation of atherosclerosis it needs to go through peroxidation by ROS (31). This step is inhibited by endogenous antioxidants. It is also possible for other antioxidants such as those found in olive oil to limit this process, which can help arrest the formation of atherosclerosis (30).

Apart from being a source of polyphenols, olive oil and the MD as a whole, contains a large amount of monounsaturated fatty acids compared to polyunsaturated fatty acids (26). A meta-analysis from 2019 investigated the correlation of CVD and dietary fatty acids. It demonstrated an association between increased dietary intake of TFA and risk of CVDs. However, it did not find any correlation with dietary intake of MUFA, SFA, PUFA, total fat intake and the risk of CVDs. Studies followed up for more than 10 years found a cardio-protective effect of PUFA in a subgroup analysis (32).

1.2.3 Fruits and vegetables

It is common knowledge that a healthy diet should consist of daily consumption of fruits and vegetables. This has been a part of the well-known food triangle for decades (33). Each fruit and vegetable vary widely in its nutritious contents, but they are rich resources of many vitamins, minerals, fibers and antioxidants (34). A meta-analysis from 2016 included 95 studies and 142 publications, showed a decreased risk and mortality of CVDs with consumption of fruits and vegetables up to 800g a day (35).

1.2.4 Nuts and legumes

Common nuts consumed today are walnuts, almonds, hazelnut, Brazil nuts and cashew. Nuts is a very nutrient-dense food containing fatty acids (MUFA, PUFA), vitamins, antioxidants phytosterols, fibers, folate, thiamine, phenolic compounds to name a few (36-38). A study reviewing RCT in the past 25 years showed a notable relation between CVDs (fatal and non-fatal), and nut intake. The proposed mechanism involves the nutrients effects on inflammation, oxidative stress, endothelial function, cholesterol lowering, and possibly non-alcoholic fatty liver disease (NAFLD) and gut microbiota, (table 2) (37, 38). A meta-analysis of moderate quality observed a decreased risk of stroke with a daily intake of 12g a day (39). Walnuts in particular has shown great promise of health benefits in relation to CVDs (40).

Table 2. Overview of the proposed mechanism's nuts ameliorate cardiovascular, gut and metabolic health

Component	Proposed mechanism
Cholesterol metabolism	Reduced cholesterol absorption • Inhibition of HMG-CoA reductase • Increased bile acid production • Stimulation of 7 α-hydroxylase • Increased cholesterol efflux • Reduced cholesterol ester production
Vascular function and blood pressure	Modulation of nitric oxide production • Reduced vascular inflammation • Reduced LDL oxidation • Inhibition of platelet aggregation • Reduced oxidative stress
Inflammation	Reduced production of pro-inflammatory cytokines
Fatty liver	Increased fatty acid β-oxidation • Reduced oxidative stress and inflammation • Reduced fibrogenesis
Gut microbiome	Altered gut microbiota profile

Abbreviations: HMG-CoA- β-Hydroxy β-methylglutaryl-CoA**; LDL-** low-density lipoprotein.

Source: Coates AM, Hill AM, Tan SY. Nuts and Cardiovascular Disease Prevention. Curr Atheroscler Rep. 2018;20:48.

Legumes is a group of food from plants that produce seeds. Commonly legumes found in diets around the world include peanuts, beans, soybeans, chickpeas and lentils. They stand as a source of a wide variety of nutrients such as vitamins, protein, PUFA, fibers, antioxidant phytochemicals and carbohydrates with a low glycemic index (41, 42). A meta-analysis conducted in 2017 showed a reduced all-cause mortality in association with legume intake. However further research is required due to insufficient relevant studies (43).

1.2.5 Fiber

As mentioned, dietary fibers are polymers of carbohydrates i.e., oligo- and polysaccharides. In contrary to macronutrients, they are not digested but rather fermented in your gut. Because of this some dietary fibers act as prebiotics, contributing to the gut microbiota by changing its activity and composition. The microbiota consists of close to one thousand species of bacteria with a colony-forming unit of 10^{11}–10^{12} per gram (44). It is a very complex topic which we do not fully understand (45). It develops through your whole life and has many important functions. Aside from being an integral step of your innate immune system it also helps control the enteric nervous system (ENS) and central nervous system (CNS), participates in digestion, plays a role in obesity, diabetes and inflammatory bowel disease (IBD) to name a few (45-49). Another meta-analysis showed an inverse correlation between total mortality and dietary fiber intake (50). Today more research is needed to further understand the role if dietary fiber and its effect on gut microbiota (51).

1.3 Physical activity and its health impacts

Through the years physical activity has proved to offer many health benefits related to somatic and mental illness (52-57). Research shows that active compared to inactive people are in a better health condition, physical function and live longer lives with increased healthy years of life (Figure 2) (52). Daily physical activity helps to prevent primary and secondary CVDs (54-57).

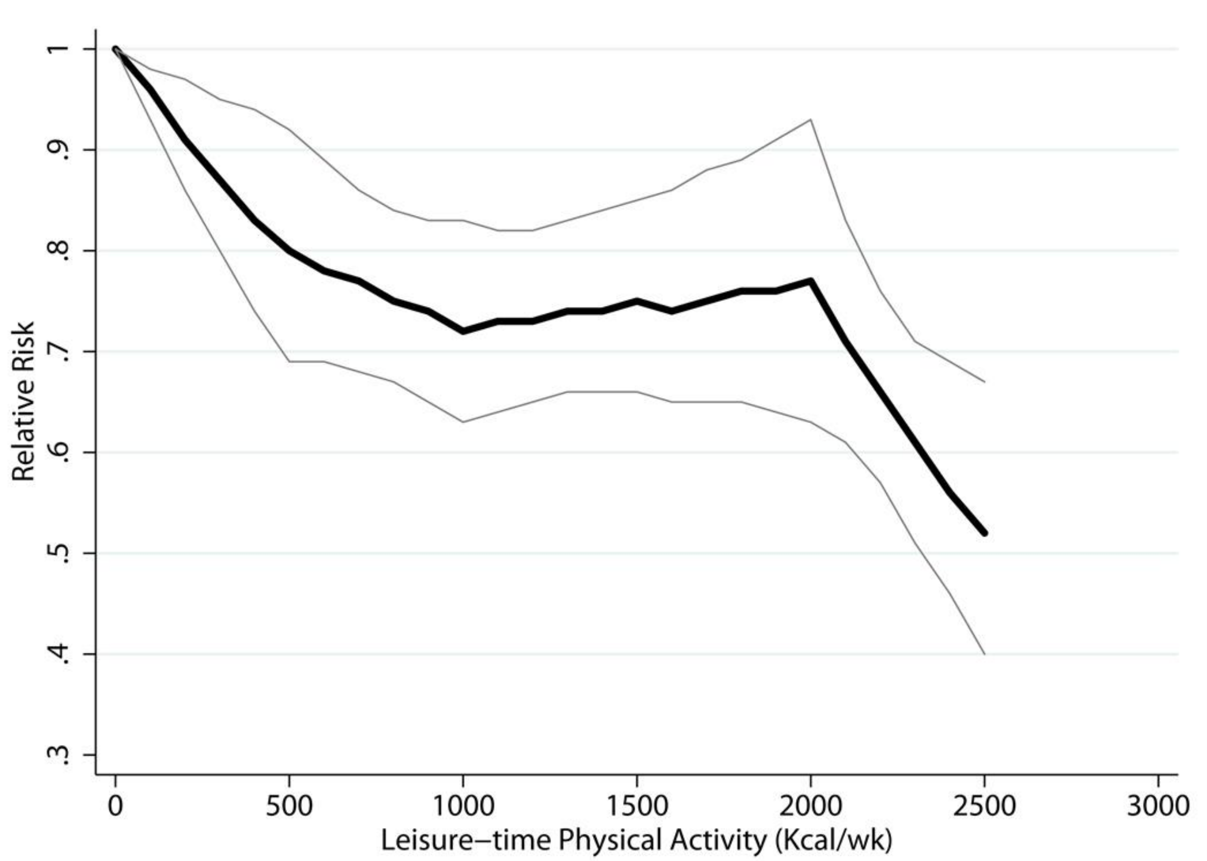


Figure 2. Relative Risks of coronary heart disease. Source: Sattelmair J, Pertman J, Ding EL, Kohl HW 3rd, Haskell W, Lee IM. Dose response between physical activity and risk of coronary heart disease: a meta-analysis. Circulation. 2011;124:789-95.

One study saw maximal risk reduction of all-cause mortality with light intensity activity for 375 min/day or moderate to vigorous activity intensity for 24 min/day (52). Portrayed in Figure 3 below.

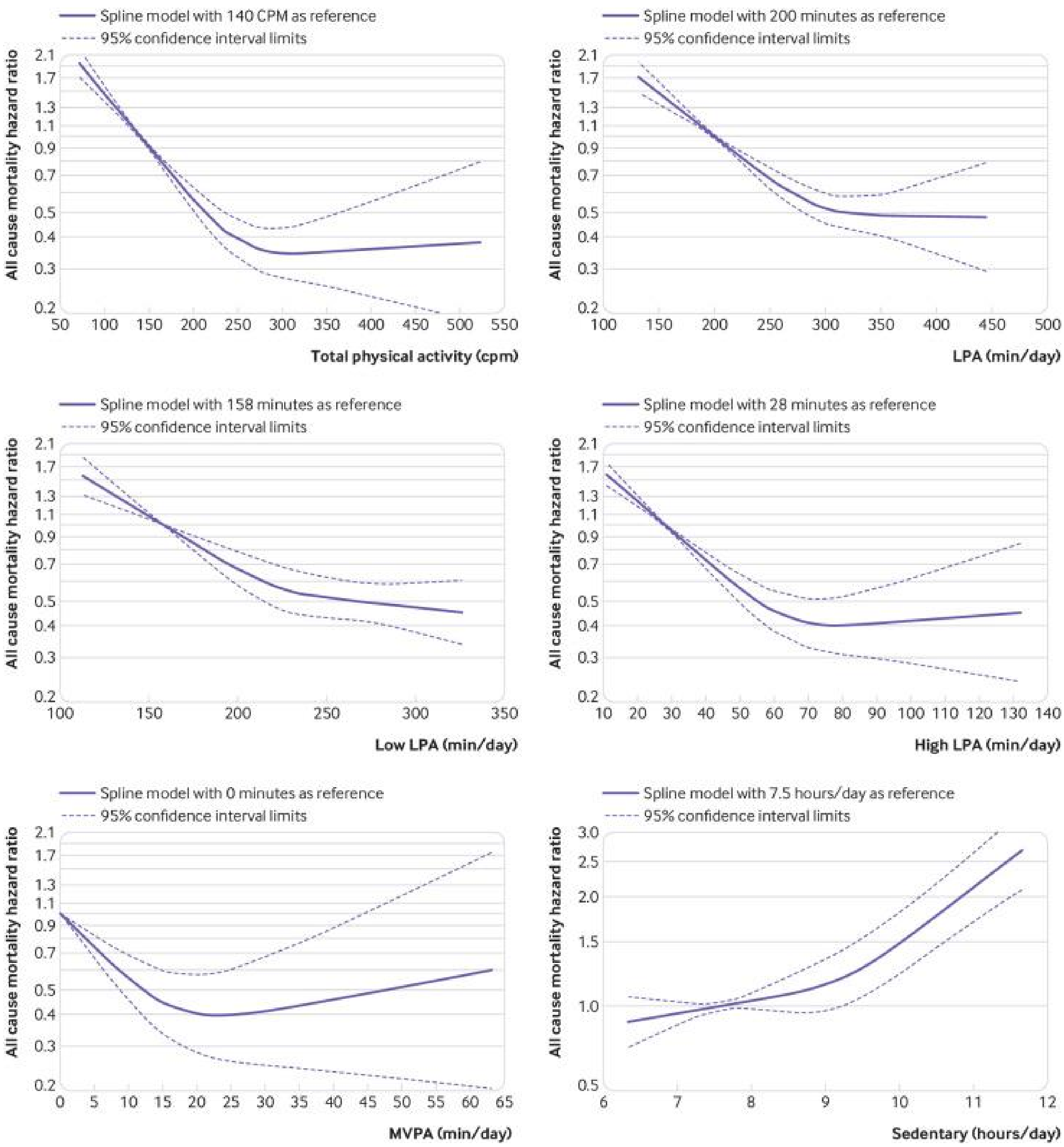


Figure 3. Dose response relationship between different levels of physical activity, sedentary time and all-cause mortality. Source: 1. Ekelund U, Tarp J, Steene-Johannessen J, Hansen BH, Jefferis B, Fagerland MW, et al. Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: systematic review and harmonised meta-analysis. BMJ. 2019;366:l4570.

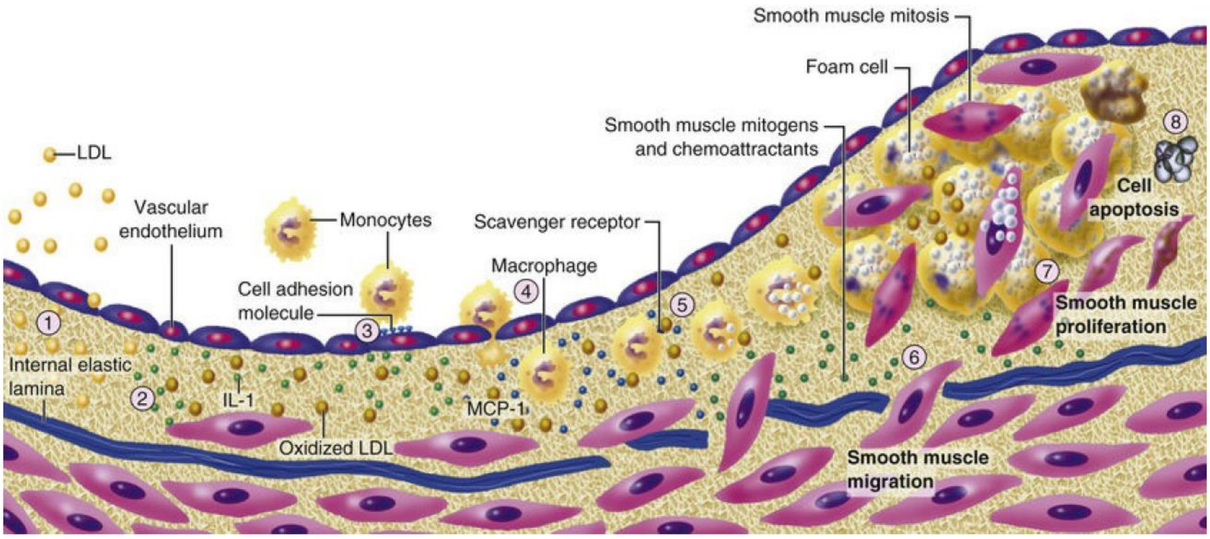
Physical activity or exercise can be categorized according to how the ATP required for muscle contraction is achieved. ATP is stored within the muscle, but this is very limited and exhausted in a short manner of time. Thereafter the supply of ATP is dependent on two pathways, aerobic and anaerobic. The aerobic pathway utilizes oxygen delivered by the cardiovascular and respiratory systems to produce ATP in the mitochondria. Typical aerobic exercises include running, cycling and swimming. The anaerobic pathway activates when the demand for ATP is higher than the aerobic pathway can supply. ATP is produced by breaking down glycogen and phosphocreatine into lactate. Anaerobic activities are activities requiring a lot of strength in a short amount of time. Examples of typical anaerobic exercises are sprint, weightlifting, interval training with high intensity. In general, any aerobic activity can become anaerobic if there is enough intensity (58).

1.4 Pathogenesis of atherosclerosis

The arterial wall is composed of 3 layers, from the outer layer tunica externa, tunica media, and the innermost layer tunica intima, which encircles a basement membrane. One of the first steps is migration and accumulation of lipoproteins from the blood into to the intima (figure 4). Here they interact with proteoglycans resulting in entrapment, accumulation, as well as becoming more susceptible to oxidative stress and chemical reactions. In the newly formed atheroma benefactors to increased oxidative stress includes, infiltrating leukocytes and myeloperoxidase expressing lipoxygenases, vascular cells expressing reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases.

Leukocyte adhesion and migration through the endothelium is normally resisted. In hypercholesterolemia however increased expression of selectins and especially vascular cell adhesion molecule 1 (VCAM-1) which interacts with very late antigen 4 (VLA-4) allows monocytes to enter the intima, where they take up the lipoproteins and evolve into foam cells. Following adhesion, the migration of leukocytes is facilitated by various chemokines like monocyte chemoattractant protein 1 (MCP-1). Once inside the intima, macrophage colony-stimulating factor increases the number of scavenger receptors on the macrophages. These foam cells have a proinflammatory nature by several mediators and oxidative species which further promotes the formation of the plaque. Both innate and adaptive immunity are believed to play a role in the progression of atherosclerosis. Eventually smooth muscle cells (SMC) migrate into the intima and starts to proliferate. This is thought to be in a burst like fashion with apoptosis and replication occuring in parallel. SMCs by stimuli of factors such as transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF) produce extracellular matrix (ECM), namely proteoglycans, collagen and elastin. The atheroma continues to grow by the help of its own microcirculation and becomes calcified. Finally, the fatty streak has become a with a lipid core and a fibrous cap (figure 4) (59).

It has been proposed that some dietary nutrients and bioactive compounds can limit some or several aspects of atherosclerosis formation. Their activity is linked, among other things, to a decrease in inflammatory response, antioxidant capability to prevent LDL particle oxidation, migration of leukocytes, adhesion molecules, vascular smooth muscle cell (VSMC) viability, and blood pressure lowering. Additionally, recent research has demonstrated that the microbiota may have a significant role in CVD risk through the creation of certain metabolites that may control directly or indirectly the development of atherosclerotic plaques (60).



LDL- low-density lipoprotein; **IL-1-** interleukin 1

Figure 4. Formation of atherosclerotic plaque, the initiation and progression. Source: Zipes D. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 11th ed. Philadelphia: Elsevier; 2019. p. 2230

1.5 Diet and healthy eating in athletes

Athletes have increased energy requirements due to their relative higher energy expenditure and extra liquid to replenish sweat losses. Not only that but adequate intake of nutrients and bioactive compounds is also vital to maximize exercise effects and performance. There has been many studies on how much of the respective macronutrients and micronutrients they should consume on a daily basis for maximum effect in respect to their physical exercises. Not all athletes have the same exact requirements. It depends on the type and amount of exercise, body composition, sex, age, etc. (61-64). For example, endurance athletes have a varying degree of daily carbohydrate requirements, depending on the type and length of exercise, and if the athlete is preparing for a competition. They could be anywhere from 6 to 10g/kg a day (61). In resistance training, the goal is to stimulate muscle growth and consuming adequate amount of protein is an essential part. The International Society of Sports and Nutrition recommends most exercising people a daily intake of 1.4 - 2g/kg high quality protein, preferably from whole foods but supplemental protein is also adequate. Apart from quantity and quality, what time during the day you consume the protein can have an additional effect, namely before sleep (62). Fats has not gained the same attention as the two other macronutrients. However, it is of course also a very important nutrient and one can experience deficiencies of carotenoids, vitamins and essential fatty acids if they make up less than 20% of the overall energy. Conjugated linoleic acids (CLA) could potentially also become deficient. CLA has several proposed benefits, such as testosterone synthesis, reduced lipid uptake from adipocytes and may impede the development of atherogenesis and cancer (61).

Another field of interest is micronutrient supplementation. As mentioned previously in the text they are vital compounds for many physiological activities in the human body (9-12). They are commonly used today to gain several supposed benefits, e.g. improving performance and recovery. Despite its popularity, to this date there is still lacking any significant evidence that such supplementation would prove beneficial unless there is an obvious deficiency. On the contrary micronutrient supplementation could prove harmful because of its potential to disturb certain physiological reactions (63). The best evidence for improving exercise capacity and athletic performance comes from studies on iron and magnesium supplements (64).

2. OBJECTIVES

The main aim of this study was to compare adherence to Mediterranean diet and nutritional habits between male and female fitness center users. In addition, we wanted to evaluate the adherence to the specific dietary components of the Mediterranean diet.

Hypotheses:

1. Mediterranean diet serving score (MDSS) will be higher in female fitness center users than in male counterparts.
2. The percentage of participants adhering to the Mediterranean diet will be greater in population of female fitness center users in comparison to male population.
3. There will be no difference in following eating guidelines for specific dietary components of the Mediterranean diet between female and male fitness center users.
4. Duration of using a fitness center and time spent in a fitness center will independently predict adherence to Mediterranean diet.

3. SUBJECTS AND METHODS

3.1. Study setting and participants

This is a cross-sectional study that included a total of 405 fitness centre users from city of Split, Croatia. A link to anonymous online Google Forms® survey was distributed via e-mails and social groups from July to October 2021. All of the participants were 18-65 years old fitness centre users that were active for at least 3 months, for minimally 1 times per week training. Users that were involved in professional sport were excluded from the study (receiving payment, participation in professional tournaments, training for more than 6 times per week).

Ethics Committee of University of Split School of Medicine approved the protocol of this study (No: 003-08/20-03/0005), and it was conducted according to the Declaration of Helsinki. Finally, submission of all of the answers in Google Forms® survey application was considered as informed consent, that was emphasized to participants as well.

3.2. Survey

Comprehensive survey consisted of two main parts, whereas first one collected general information, including gender, age, training characteristics, anthropometric measurements and usage of dietary supplements. These 12 exact items were developed after rigorous literature review at Department of Pathophysiology, University of Split School of Medicine.

Second part of the questionnaire investigated overall adherence, as well as adherence to individual components of Mediterranean diet through MDSS. It is a 14-item scale, validated in Croatian language that is based on Mediterranean Diet Pyramid (65, 66). If guidelines for consummation of certain food groups were satisfied, then a score of one, two or three points were given for each item/food group. Hence, most important food groups for MD, including vegetables, olive oil, cereals and fruits were given three points; dairy products and nuts were given two points; while red and white meat, fish, potatoes, wine, sweets, eggs and legumes were given one point. Participants with total MDSS score ≥14 points were considered to adhere to MD.

3.3. Statistical analysis

All statistical analyses were performed using MedCalc for Windows (MedCalc Software, Ostend, Belgium, version 17.4.1). Normality of data was evaluated using the Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation or median (interquartile range), as appropriate considering normality of distribution. Categorical variables were presented as a whole number (N) and percentage (%). For determining differences between the groups, an independent samples t-test was used for continuous variables with normal distribution, whereas the Mann–Whitney U test was used for continuous variables with non-normal distribution. The chi-square ($\chi 2$) test was used to determine differences between groups in terms of categorical variables. the independent predictors for adherence to the MD were evaluated with multivariable logistic regression, with the OR (odds ratio), 95% CI (95% confidence interval) and p-value reported. The level of statistical significance was set at p-value < 0.05 for all comparisons.

4. RESULTS

The study included a total of 405 participants, with 193 and 212 males and females, respectively. The males where significantly younger compared to the females (28.2 ± 8.0 vs. 32.8 ± 10.6, P<0,001), and consume more dietary supplements (63.7 vs. 49 %, P=0.004), particularly whey protein (77.7 vs. 47.6 %, P<0.001) and creatine (25.3 vs. 3.8 %, P<0.001). All parameters describing main characteristics of study population are presented in Table 3.

Table 3. Main characteristics of the study sample and differences regarding gender.

Parameter	**Study population N=405**	**Males N=193**	**Females N=212**	***p****
Age (years)	29.1 ± 8.8	28.2 ± 8.0	32.8 ± 10.6	<0.001
Weight (kg)	79.3 ± 15.6	87.3 ± 12.2	68.9 ± 12.5	<0.001
Height (cm)	179.6 ± 9.5	184.8 ± 7.3	171.7 ± 5.8	<0.001
BMI (kg/m^2)	24.4 ± 3.4	25.4 ± 2.5	23.4 ± 4.6	<0.001
Using dietary supplements (N, %)				
Yes (N, %)	227 (56.0)	123 (63.7)	104 (49.0)	0.004
No (N, %)	178 (44.0)	70 (26.3)	108 (51.0)	
Dietary supplements used				
Whey protein (N, %)	251 (61.9)	150 (77.7)	101 (47.6)	<0.001
BCAA (N, %)	132 (32.7)	65 (33.6)	67 (31.5)	0.476
Creatine (N, %)	57 (14.0)	49 (25.3)	8 (3.8)	<0.001
Magnesium (N, %)	144 (35.6)	72 (37.3)	72 (34.0)	0.549
Vitamin C (N, %)	287 (70.9)	132 (68.4)	155 (73.1)	0.350
Vitamin B complex (N, %)	59 (14.6)	26 (13.5)	33 (15.6)	0.648
Multivitamin (N, %)	129 (32.1)	68 (28.0)	60 (38.4)	0.365
Duration of using a fitness center				
< 1 year (N, %)	137 (33.8)	65 (33.7)	72 (34.0)	0.985
1 – 3 years (N, %)	96 (23.7)	46 (23.8)	50 (23.6)	
4 – 7 years (N, %)	96 (23.7)	47 (24.4)	49 (23.1)	
> 7 years (N, %)	76 (18.8)	35 (18.1)	41 (19.3)	
How many days a week do you averagely exercise?				
1-2 days	84 (20.8%)	33 (17.1)	51 (24.1)	0.225
3-4 days	244 (60.3%)	118 (61.1)	126 (59.4)	
5-7 days	77 (19.0%)	42 (21.8)	35 (16.5)	
How long does your average exercises last?				
< 45 minutes	46 (11.4)	28 (14.5)	18 (8.5)	0.155
45-90 minutes	239 (81.5)	152 (78.8)	177 (83.5)	
> 90 minutes	30 (7.4)	13 (6.7)	17 (8.0)	

All the data is presented as whole numbers (percentage) or mean ± SD.
Abbreviations: BMI- body mass index; **BCAA**- Branched-chain amino acids.
* chi-square test or student t-test

MDSS analysis have shown that investigated population most commonly followed eating guidelines for potatoes (90.1%) and white meat (91.4%). Furthermore, when comparing adherence to the MDSS components between the genders, the results have shown that more females where compliant to the recommendations for dietary intake of sweets (58.5 vs. 41.5 %, P<0.001) and dairy products (19.3 vs. 11.9 %, P=0.056). Regarding other MDSS components, study population did not show statistical differences in following the eating guidelines between the genders (Table 4).

Table 4. Adherence to MDSS components in the study sample and differences regarding genders.

Parameter	**Study sample N=405**	**Males N=193**	**Females N=212**	***p****
Cereals (N, %)	87 (21.5)	41 (21.2)	46 (21.7)	0.992
Potatoes (N, %)	365 (90.1)	173 (89.6)	192 (90.6)	0.883
Olive oil (N, %)	59 (14.6)	28 (14.5)	31 (14.6)	0.913
Nuts (N, %)	124 (30.6)	65 (33.7)	59 (27.8)	0.243
Fish (N, %)	240 (59.3)	120 (62.2)	120 (56.6)	0.299
White meat (N, %)	370 (91.4)	176 (91.2)	194 (91.5)	0.949
Red meat (N, %)	73 (18.0)	29 (15.0)	44 (20.8)	0.171
Fruits (N, %)	70 (17.3)	30 (15.5)	40 (18.9)	0.452
Vegetables (N, %)	89 (22.0)	35 (18.1)	54 (33.4)	0.096
Dairy (N, %)	64 (15.8)	23 (11.9)	41 (19.3)	0.056
Legumes (N, %)	311 (76.8)	143 (74.1)	168 (79.2)	0.267
Eggs (N, %)	222 (54.8)	101 (52.3)	121 (57.1)	0.390
Sweets (N, %)	204 (50.4)	80 (41.5)	124 (58.5)	<0.001
Wine (N, %)	1 (0.2)	0 (0)	1 (0.5)	0.962

All the data is presented as whole numbers (percentage).
* chi-square test or Fisher's exact test

Further MDSS analysis have shown that median MDSS score was significantly lower in men when compared to women (7.0 (5.0-8.25) vs. 7.0 (6.0-10.0), P=0.030) (Figure 5). However, out of the 405 participants, 19 males (9.8%) and 32 females (15.1%) where overall adherent to MD, without statistical significant difference between the genders (P=0.149) (Figure 6).

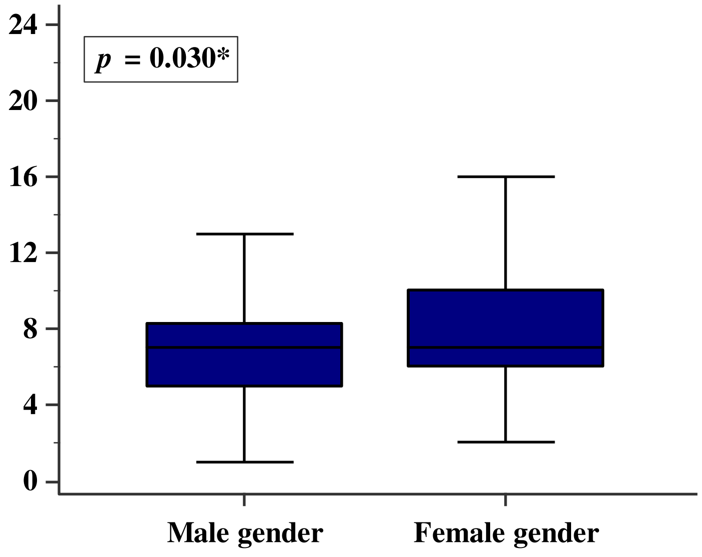


Figure 5. Comparison of the total MDSS score between the male gender (N=193) and the female gender (N=212) subjects.
* Mann-Whitney U test

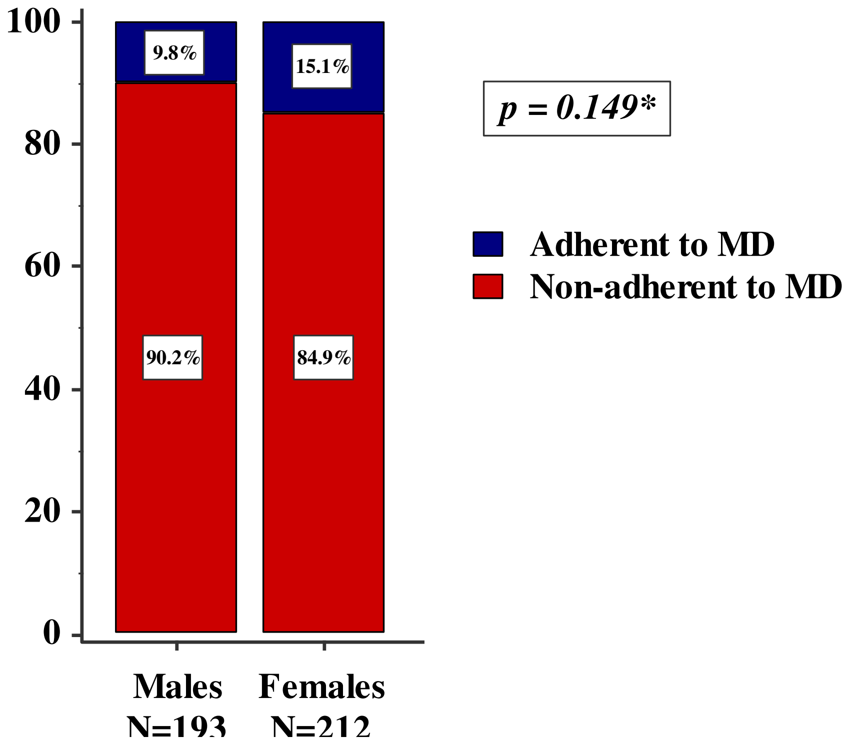


Figure 6. Difference in the adherence to the MD (MDSS score ≥ 14) between the male gender and the female gender subjects.
* chi-square test

In order to determine independent variables associated with positive adherence to MD, a multivariable logistic regression model was used. None of the parameters proved to be of statistical significance. Detailed logistic regression model can be seen in Table 5.

Table 5. Multivariable logistic regression model showing independent predictors for the positive adherence to MD (MDSS score ≥ 14).

Variable	**OR**	**95% CI**	***P***
Age	1.007	0.975 to 1.041	0.649
Female gender*	1.560	0.792 to 3.070	0.197
BMI	1.006	0.925 to 1.094	0.884
Dietary supplements usage†	0.854	0.464 to 1.571	0.612
Duration of using a fitness center	1.012	0.817 to 1.253	0.910
Days/wk using a fitness center	0.831	0.646 to 1.068	0.149

Abbreviations: MD- Mediterranean diet; **OR**- multivariable adjusted odds ratio; **95% CI**- 95% confidence interval; **BMI**- body mass index.
* Male gender is the reference group
† Dietary supplements non-users are the reference group

5. DISCUSSION

In this study all of the participants were fitness center users and living in Split, Croatia. Around half (49%) of the females and 63% of the males were taking dietary supplements. The most common supplement with statistical significance was whey protein and it was especially frequent among the males. Protein is a key component for muscle growth and current recommendations suggest a daily intake of at least 1.4g for most exercising people and whey protein is a very convenient supplement to help reach those requirements (62). A similar study investigating dietary supplements among male fitness center users in Riyadh, Saudi-Arabia, also found protein to be the most common supplement (67). Both studies included adult fitness center users, but in a completely different region than ours, further establishing fitness center users' nutritional habits toward supplementation, more specifically protein supplementation. Additionally, supplement users were considerably more likely to have positive attitudes on dietary supplements from a health perspective than non-users (67). It's possible that fitness center users, apart from utilizing whey to gain adequate protein intake for optimal muscle growth, believe in other positive effects toward exercise. A study from 2017 investigated whey proteins effect for resistance exercise and concluded that it can increase whole body anabolism and might improve fast recovery from exercise (68).

Only 15.1% and 9.8% of the female and male participants respectively was adherent to MD, but the results were not statistically significant. However, it was statistically significant that females had a higher overall MDSS score. As for the specific components, most adherence was towards potatoes (90.1%), white meat (91.4%), and legumes (76.8%) but the only parameter of statistical significance was sweets (58.5% females, 41.5% males, $P<0.001$). Across the spectrum then, females may appear to have a higher adherence to MD in comparison to males. However, the data acquired from this study is not sufficient to make any conclusion on that claim. A recent study also investigating MD adherence, but in the Portuguese population, found females to have a significantly higher adherence compared to males, and similar to our results only 17.1% of the participants had high adherence (69). Resemblance between the studies includes the participants age and their residence along the Mediterranean. However, the majority of their participants did not engage in regular physical activity. The same study also discovered higher adherence among people who had jobs and eating more meals per day.

Both physical activity and the Mediterranean diet has been thoroughly studied for many years and has proven to offer a vast array health benefits and helps to prolong healthy living (19-24, 52-57). It seems logical to assume that people who wants to improve their health, for example a fitness center user, would be more compliant to healthy diets like MD. A study investigating MD adherence in Spanish university students discovered that those who adhered to MD and were in good physical shape had the healthiest cardiometabolic profiles (70). In a different study involving teenage Icelandic students, it was discovered that those who adhered to the Mediterranean diet to a medium or high degree performed best on measures of physical fitness related to their health (71). These studies may reflect a connection between the MD and physical exercise. Additionally, a study performed on young Italian adults found the participants to have a good knowledge about the diet, 23.1% had high adherence and a lower level of adherence was observed in people who do not exercise often. Although all of these studies may differ in the same population observed, indeed there seems to be a positive correlation between MD and physical exercise (72).

Perhaps one would expect the adherence to be higher especially in those who perform regular physical activity. Most European countries appear to be drifting away from MD since the mid nineteen hundred, with the exception of the northern countries where the diet is gaining popularity (73). According to recent data there seems to be a globalization of the food market where fresh ingredients and meals are being replaced by readily available processed foods (74). This might become a challenge in the future with further globalization and younger generations forgetting about their traditional eating habits. In order to improve the adherence among the population we should focus on educating the population of the diets content and health benefits.

As this is a cross-sectional study, causality between the obtained results cannot be determined. Furthermore, participants were included from only one city in Croatia, that means that there could exist regional differences in MD adherence, or that some factors that are specific for Split interfered with the results. Finally, participants could have given subjective answers that are not totally correct, as main tools were self-administered questionnaires.

6. CONCLUSION

1. The median MDSS score was significantly higher in women when compared to men.
2. In this study 9.8% males and 15.1% females where overall adherent to MD, without statistical significant difference between the genders.
3. Females were more compliant to the recommendations for dietary intake of sweets and dairy products.
4. Other independent predictors for positive adherence such as duration and time spent in fitness centers could not be determined.
5. A majority of fitness center users are using some dietary supplementation, most commonly whey protein.

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8. SUMMARY

Objectives: To investigate nutritional habits and attitudes in male and female fitness center users, as well as the degree of adherence to the Mediterranean diet (MD) and its components.

Materials and Methods: This was a cross-sectional study, including 405 fitness center users in Split, Croatia, conducted from July to October 2021. All of the participants were active fitness center users between the ages of 18 and 65 and trained at least once a week, professional athletes were excluded from the study. Online Google Forms® survey was distributed via e-mails and social groups and it consisted of two main parts, with general information and dietary supplements information collection in first, and adherence to MD and its components using Mediterranean Dietary Serving Score (MDSS) in second.

Results: The study included 193 male and 212 female fitness center users. The males consume more dietary supplements when compared to females (63.7 vs. 49 %, $P=0.004$), including whey protein (77.7 vs. 47.6 %, $P<0.001$) and creatine (25.3 vs. 3.8 %, $P<0.001$). Furthermore, MDSS analysis have shown that investigated population most commonly followed eating guidelines for potatoes (90.1%) and white meat (91.4%), with significantly more females where compliant to the recommendations for dietary intake of sweets (58.5 vs. 41.5 %, $P<0.001$). Further MDSS analysis have shown that median MDSS score was significantly lower in men when compared to women (7.0 (5.0-8.25) vs. 7.0 (6.0-10.0), $P=0.030$). Moreover, 19 males (9.8%) and 32 females (15.1%) where overall adherent to MD, without statistical significant difference between the genders ($P=0.149$). Finally, a multiple logistic regression model that was used to determine independent variables associated with positive adherence to MD showed that none of the investigated parameters proved to be of statistical significance.

Conclusion: Majority of fitness center users are using some dietary supplementation, most commonly whey protein. Overall adherence to Mediterranean diet was low. Females have a higher total MDSS score and no significant difference in adherence was observed between the genders.

9. CROATIAN SUMMARY

Naslov: Prehrambene navike i stavovi o prehrani korisnika fitness centara

Ciljevi: Istražiti prehrambene navike i stavove o prehrani muških i ženskih korisnika fitness centara, kao i stupanj pridržavanja mediteranske prehrane (MP) i njenih komponenti.

Materijali i metode: Ovo je presječna studija, koja je uključila 405 korisnika fitness centara u Splitu u Hrvatskoj, provedena od srpnja do listopada 2021. Svi su sudionici bili aktivni korisnici fitness centara u dobi od 18 do 65 godina i trenirali su barem jednom tjedno, profesionalni sportaši bili su isključeni iz studije. Online Google Forms® anketa distribuirana je e-poštom i društvenim mrežama, a sastojala se od dva glavna dijela, prikupljanje općih informacija i informacija o dodacima prehrani u prvom, te o pridržavanje MP-e i njezinih komponenti korištenjem *Mediterranean Dietary Serving Score* (MDSS) u drugom.

Rezultati: U istraživanju je sudjelovalo 193 muškaraca i 212 žena. Muškarci konzumiraju više dodataka prehrani u usporedbi sa ženama (63,7 naspram 49 %, P=0,004), uključujući proteine sirutke (77,7 naspram 47,6 %, P<0,001) i kreatin (25,3 naspram 3,8 %, P<0,001). Nadalje, MDSS analiza je pokazala da je ispitana populacija najčešće slijedila smjernice za prehranu krumpirom (90,1%) i bijelim mesom (91,4%), pri čemu se značajno više žena pridržavalo preporuka za prehrambeni unos slatkiša (58,5 naspram 41,5%, P <0,001). Daljnja MDSS analiza pokazala je da je medijan MDSS rezultata bio značajno niži u muškaraca u usporedbi sa ženama (7,0 (5,0-8,25) naspram 7,0 (6,0-10,0), P=0,030). Štoviše, 19 muškaraca (9,8%) i 32 žene (15,1%) općenito se pridržavalo MP-e, bez statistički značajne razlike između spolova (P=0,149). Konačno, višestruki logistički regresijski model koji je korišten za određivanje neovisnih varijabli povezanih s pozitivnim pridržavanjem MP-e pokazao je da se niti jedan od ispitivanih parametara nije pokazao statistički značajnim.

Zaključci: Većina korisnika fitness centara koristi neki dodatak prehrani, najčešće protein sirutke. Sveukupno pridržavanje mediteranske prehrane bilo je nisko. Žene imaju viši ukupni rezultat MDSS-a, a nije opažena značajna razlika u adherenciji između spolova.

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2016 – 2022	University of Split, School of Medicine
2013 – 2016	Stord vidaregåande skule

Work experience:

07-09/2021	Medical student with temporary physician’s license, Haukeland hospital
2018-2020	Summer job as a medical assistant, Stord hospital
2014-2015	Sales associate, Byggmax

Personal skills and competence:

Mother tongue:	Norwegian
Other language:	Swedish, Danish and English
Driver license:	Category B
2017 – 2021	Vice president of ANSA