**DOI:** 10.7251/QOL2401046K **UDC:** 616.441-008.64-06:616.13-004
*Original scientific paper*

# Influence of Modified Diet and Low Grade Physical Activity on Serum Lipids in Patients With Type IIa and IIb dyslipoproteinaemia

Jasmina Krehić
*Department for Clinical Pharmacology, General hospital "Prim.dr. Abdulah Nakaš" Sarajevo, FBiH, BiH, jasminakrehic@yahoo.com*

**ABSTRACT:** Dyslipoproteinaemia, a common name for different types of serum lipid disorders, plays one of the crucial roles in the etiology of atherosclerosis. Complications of atherosclerosis, primarily coronary heart disease (CHD), in early life lead to significant disability and are the worldwide leading cause of death. Treatment of dyslipoproteinaemia, as a separate disease, is carried out as a part of the treatment of CHD, and includes non-pharmacological and pharmacological measures. Recognizing an underestimated value and role of dietary modifications in everyday life of patients with hypercholesterolaemia, we decided to actualize our investigation from 2000, as we witness that lipid lowering drugs are prescribed almost as soon as elevated serum lipid levels are detected. The aim of our study was to evaluate the effects of a hypolipemic diet and low grade physical activity on serum lipid/lipoprotein levels in twenty patients with primary dyslipoproteinemia Type IIa and IIb, according to Fredrickson classification. All patients with Type IIa and IIb dyslipoproteinaemias were subjected to a hypolipemic diet that is a modification of Step I, Step II (NCEP) and the Mediterranean diet for a period of four weeks. The minimum physical activity involved a daily light walk lasting at least 1 hour. Complete lipid status has been done before and after the period of four weeks. The positive effects of our modified diet were shown as a decrease of all proatherogenic lipid/lipoprotein serum concentrations, except for β-lipoprotein. Serum concentrations of antiatherogenic α-lipoprotein increased.

**Keywords:** dyslipoproteinaemia, hypercholesterolemia, diet, exercise, serum lipids, lipoproteins.

## INTRODUCTION

### Dyslipoproteinaemia and its role in the development of atherosclerosis

Dyslipoproteinaemia - disorders of serum lipid/lipoprotein metabolism plays one of the crucial roles in the etiology of atherosclerosis, and results in clinical manifestations and complications of atherosclerosis such as coronary heart disease - CHD (angina pectoris, myocardial infarction), renal insufficiency, peripheral and central vascular diseases, dementia and pancreatitis. Complications of atherosclerosis, primarily CHD, in early life lead to significant disability and are the worldwide leading cause of death, both in total mortality and in mortality from cardiovascular diseases (Farnier et al. 1998; WHO, 2020). Although the disease has a multifactorial etiology, an increase in serum total cholesterol (TC), especially low-density lipoprotein cholesterol (LDL-C), is considered the most important risk factor in the development of atherosclerosis (Sloop, 1999; Zmysłowski and Szterk, 2017; Mortensen et al., 2023). Regardless of whether dyslipoproteinaemias are primary – genetic, or secondary (inadequate diet, poorly controlled diabetes, hypothyroidism, nephrotic syndrome, biliary obstruction, alcoholism, use of certain drugs), or combination of both, dyslipoproteinaemias represent a problem in managing patients with atherosclerosis and its complications. The need to lower serum cholesterol levels is increasingly emphasized, primarily by modifying the lifestyle, and if the target values are not achieved, then by drug therapy. Every 10% reduction in cholesterol is associated with approximately 20% to 30% reduction in the incidence of CHD (Drug Facts and Comparisons, 2000).

The recommendations of the Working Group of the Association of European Societies - European Society of Cardiology (ESC), European Atherosclerosis Society (EAS) and European Society of Hypertension (ESH) - were published in 1998 in the *European Heart Journal, Atherosclerosis and Journal of Hypertension* (Wood et al.,1998). Serum lipid target values in Europe differ from lipid target values in America. The new target values in Europe, based on *Recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention.* for cholesterol should be below 5.0 mmol/L (190 mg/dL), and for LDL cholesterol below 3.0 mmol/L (115 mg/dL). HDL cholesterol and triglyceride concentrations were not taken as a target values in hyperlipoproteinemia therapy, however HDL values below 1.0 mmol/L (40 mg/dL) and fasting triglyceride values greater than 2.0 mmol/L (180 mg/ dL) are set as markers of increased risk for CHD (Wood et al.,1998).

Disorders of lipoprotein metabolism were previously classified as hyperlipoproteinemias and were defined as an increase in lipoprotein levels above the values that are average for 95% of the population, that is, they were defined as values within two standard deviations (SD) above or below the mean value for the population (Steinberg and Gotto, 1999). Today, the classification according to Fredrickson and colleagues, recognized by the World Health Organization (WHO), is still in use (Beaumont, 1970; WHO 2021). This classification phenotypically categorizes 5, actually 6 types of hyperlipoproteinemias (Type I, IIa, IIb, III, IV and V ) only according to which lipids and lipoproteins are elevated, regardless of etiology. WHO Fredrickson classification of lipid disorders is associated with clinical disorders (Table 1).

**Table 1.** Fredrickson classification of primary hyperlipidemias

| Hyperlipoproteinemia | Name of disease | Elevated lipoprotein | Elevated lipid fraction |
|---|---|---|---|
| Type I | Hyperchylomicronemia | Chylomicrons | Triglycerides ++ |
| Type IIa | Familial hypercholesterolemia | LDL | Cholesterol++ |
| Type IIb | Familial combined hypercholesterolemia (hyperlipidemia) | LDL and VLDL | Cholesterol ++ and triglycerides + |
| Type III | Dysbetalipoproteinemia | IDL | Triglycerides + and cholesterol + |
| Type IV | Familial hypertriglyceridemia | VLDL | Triglycerides ++ Cholesterol N+ |
| Type V | Familial lipoprotein lipase deficiency | VLDL and Chylomicrons | Triglycerides ++ and cholesterol + |

+ = increased; ++ = greatly increased; N= normal; N+ = normal or increased (adapted from Chandra et al.,2014; WHO, 2021)

Cholesterol, triglycerides and phospholipids are the main lipids in the body. They are transported in the plasma bound to special proteins - apolipoproteins (apoproteins), forming hydrophilic lipoprotein complexes. They are classified into five main groups. Classification is made by size, density, electrophoretic mobility, as well as lipid and protein content (Table 2).

**Table 2.** Main lipoprotein characteristics

| Lipoprotein | Density,g/dL | Mol. mass, kDa | Diameter, nm | Content of lipids, % | | |
|---|---|---|---|---|---|---|
| | | | | TG | C | PL |
| chylomicrons | 0.95 | $400 \times 10^3$ | 75–1200 | **80–95** | 2–7 | 3–9 |
| VLDL | 0.95–1.006 | $10–80 \times 10^3$ | 30–80 | **55–80** | 5–15 | 10–20 |

| | | | | | | |
|---|---|---|---|---|---|---|
| IDL | 1.006–1.019 | 5–10 × $10^3$ | 25–35 | 20–50 | 20–40 | 15–25 |
| LDL | 1.019–1.063 | 2.3 × $10^3$ | 18–25 | 5–15 | **40–50** | 20–25 |
| HDL | 1.063–1.210 | 1.7–3.6 × $10^2$ | 5–12 | 5–10 | 15–25 | 20–30 |

VLDL - very low-density lipoprotein
IDL – intermediate-density lipoprotein
LDL – low-density lipoproteins (with subgroups $LDL_1$,$LDL_2$)
HDL – high-density lipoproteins (with subgroups $HDL_2$, $HDL_3$iHDL-C)
TG - triglycerides
C – Sum of free and esterified cholesterol
PL, phospholipids (the rest of the percentage content of lipoproteins is made up of apoproteins).

Based on localization in relation to serum proteins in electrophoretic separation, lipoproteins are divided into α-lipoproteins, pre-β-lipoproteins, β-lipoproteins, while chylomicrons lag behind at the site of application.

## Treatment of dyslipoproteinaemia

Treatment of dyslipoproteinaemia, as a separate disease, is carried out as a part of the treatment of CHD, and includes non-pharmacological and pharmacological measures.

***Diet*** is the treatment of first choice in non-pharmacological treatment of elevated serum cholesterol values, both in patients suffering from primary, and in those suffering from secondary disorders of lipid metabolism. It is based on reducing the intake of saturated fatty acids from food of animal origin, increasing the intake of fruits, vegetables and fish (Dwyer, 1997; Diab, Dastmalchi, Gulati and Michos, 2023). The main goal of diet, as a therapy, is to reduce the elevated level of cholesterol in the serum, while at the same time, maintaining an adequate composition of food. According to NCEP *Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults* (1994), diet therapy consists of two phases, Step I and Step II. Step I diet implies intake of saturated fatty acids in an amount that corresponds to 8 to 10% of total calories, 30% and fewer calories from total fats and cholesterol less than 300 mg/day. If the desired results are not achieved with the diet in Step I, it is switched to Step II, which implies intake of saturated fatty acids in an amount less than 7% of total calories and cholesterol less than 200 mg/day. In some patients, restriction of total fat intake causes a decrease in HDL-C and an increase in serum triglycerides (Knopp et al. 1997). A decrease in the concentration of HDL-C is undesirable, especially when the values in the serum are less than 0.9 mmol/L (35 mg/dl). This phenomenon is primarily a consequence of increased intake of carbohydrates and, most likely, increased intake of polyunsaturated fatty acids. Monounsaturated fatty acids lower the level of LDL-cholesterol without affecting the level of HDL-cholesterol.

An alternative strategy is the “Mediterranean diet”, which keeps the total fat intake at 35-40% of the total calorie intake, but replaces saturated fats with monounsaturated, such as those found in olives, peanuts, avocados and their oils. This diet is equally effective in lowering LDL-C but is less likely to reduce HDL-C (Lorgeril et al., 1999). Due to the significant fat intake, this diet does not lead to weight loss, so a low-fat diet is still recommended for overweight patients with dyslipoproteinaemia.

However, there are still contradictory views on which sources of certain types of fatty acids of plant origin (sunflower, soybean, olive or tropical oils) would satisfy the body’s needs, without having a harmful effect on the level of total cholesterol and triglycerides in the serum.

Adequate ***physical activity***, in addition to diet, is considered an essential element in the non-pharmacological treatment of elevated serum cholesterol concentrations. Both, weight reduction and physical activity, not only affects the reduction of LDL-cholesterol, but also causes a decrease in the level of triglyc-

erides, an increase in HDL-cholesterol, affects the reduction of high blood pressure, and reduces the risk of diabetes (Pescatello, Murphy and Costanzo, 2000; Williams and Thompson, 2013; Mann, Beedie, and Jimenez, 2014). Therefore, the risk of CHD is reduced in several ways.

### Problem statement

Until the late 1990s, when hypolipemic drugs known as statins, were for the first time approved in Bosnia and Herzegovina, different types of hypolipemic diets were recommended to patients with dyslipoproteinaemias. Hypolipemic diet, which would be acceptable and understandable for all patients who need to adhere to this way of eating, has has not been developed. Recommendations such as "total cholesterol intake should be less than 300 mg per day" represent ambiguity for the patient, i.e. makes it difficult for them to be motivated to adhere to the recommendations within this diet. So, we made a new recommendations as a "modified diet" combining Step I, Step II and Mediterranean hypolipemic diet. The aim of our study was to evaluate the influence of modified Step I, Step II and Mediterranean diet, as well as low grade physical activity, on serum lipids in patients with Fredrickson Type IIa (Familial hypercholesterolemia) and IIb (Familial combined hyperlipidemia) primary dyslipoproteinaemia.

## MATERIALS AND METHODS

### Study design

The study was conducted as an open, prospective, longitudinal study during 2000, in the settings of real life, designed as two phase trial. Phase I (duration 4 weeks) was conducted in accordance to the rules of hypolipemic drug administration, which imply that every patient must undergo a hypolipemic diet in the settins of real life, for at least 4 weeks before starting the drug therapy. Guided by these rules, all patients with Type IIa and IIb dyslipoproteinaemia were subjected to a hypolipemic diet that is a modification of Step I, Step II (NCEP) and the Mediterranean diet, adapted to the habits and social status of the population. Within the recommended dietary measures, patients were not deprived of any type of food - cereals, pasta, milk and milk products, meat, fruits,vegetables and fats. All patients, apart from verbally, also received written recommendations for a modified diet in form of individualised dietary counselling. Direct phone number, in case of any ambiguities, was available all the time for consultations. The minimum physical activity involved a daily walk lasting at least 1 hour. After 4 weeks of modified diet, all patients underwent a control status of lipid metabolism. Patients whose cholesterol values in the control status were above the laboratory reference range and above target values recommended by the Working Group of the Association of European Societies, were included in Phase II which implied the use of statins in one daily dose, at the evening and this is part of separate duiscussion.

### Subjects

After screening one hundred outpatients from the Clinic for Heart Diseases and Rheumatism of the University Clinical Centre Sarajevo, 20 drug-naïve patients with Type IIa and IIb disorders of lipid/lipoprotein metabolism, who met all inclusion criteria and none of the exclusion criteria, were included in the study. All patients voluntarily signed Informed Consent.

### Parameters

Parameters for evaluation of diet effects on serum lipid levels were: total cholesterol (TC), low-density lipoprotein – cholesterol (LDL-C), high-density lipoprotein – cholesterol (HDL-C), triglycerides

(TG), ApoB as well as very low density lipoprotein – cholesterol (VLDL-C), ApoA1, phospholipids, total lipids, lipoprotein electrophoresis, index bata/alpha, degree of lipemia, appearance of serum, TC/HDL-C ratio, atherogenic index based on LDL/HDL cholesterol ratio.

### Material and equipment

Blood samples, for analyzing serum lipid levels, were taken in the morning from the cubital vein, after overnight fasting period of at least 12 hours. Determination of lipid/lipoprotein status was performed in the Laboratory for Lipids of the Institute of Clinical Biochemistry, University Clinical Centre Sarajevo. Categorization, in accordance with Fredrickson classification, was also performed in this laboratory, by qualified specialist, in order to ensure maximum objectivity and reliability of test results. The *reference range* values in this study were the values of the Laboratory for Lipids, based on annually population statistic evaluation, while the *target values* were those recommended by European guideline form 1998. Total cholesterol, triglycerides, HDL-C, Apo AI and Apo B were determined by direct methods on the Dimension® clinical chemistry system with specific reagents. Phospholipids were also determined by the direct enzymatic method on the ABBOTT Spectrum Diagnostica apparatus. LDL-C was calculated by the Friedwald equation as follows: LDL-C mmol/L = total cholesterol –(triglycerides/2.2) – HDL-C. Lipoprotein electrophoresis was performed on cellulose acetate, while Index beta/alpha was calculated by the formula: (preβ +β)/ α. The atherogenic index was calculated as LDL/HDL cholesterol ratio.

### Statistics

The baseline values before the beginning of the modified diet, were the initial values for evaluation of the diet effects after 4 weeks. At the same time, each patient served himself as a control, so the significance of the differences in mean values for the group, between two measurements, was calculated by Student's t-test for small dependent samples (paired t-test). The percentage reduction or increase of certain parameters in the test was calculated using basic mathematical operations.

## RESULTS AND DISCUSSION

After screening one hundred patients from the Clinic for Heart Diseases and Rheumatism of the University Clinical Centre Sarajevo, 20 drug-naïve patients (11 mail and 9 femail, average age 55 years), with Type IIa (10 patients) and IIb (10 patients) dyslipidaemia were consecutively included in the study. The arrangement by type of dyslipidaemia was random. Comparations with results from other studies were intentionally done based on data from late 1990s and early 2000s, as that was the time period when our study was conducted and target values for serum lipids were published by American and European guidelines.

Analyzing the results obtained from this study, a significant decrease in total cholesterol (TC) can be observed after four weeks period of diet. The achieved TC reduction was 9.7% (P = 0.001), which correlates with the values of 7% to 16% described in young, healthy non-obese mail individuals (Jansen et al.,1998) and 8.5%, based on data from systemic overview od 19 randomised controlled trials, but achieved after 3 months of modified fat intake (Tang et al.,1998), instead after four weeks, as in our study. As our „modified diet" was a combination of Step I, Step II NCEP diet and Mediterranean diet, recorded decrease of total cholesterol values are in fact 10% what is in accordance with the data specified by Kris-Etherton and colleagues from 1988, but with difference is a quantity of cholesterol content in recommended diet. The fact is that Kris-Etherton and colleagues states that expected plasma total cholesterol (specifically low-density-lipoprotein cholesterol) reduction is approximately 10% to 20% when dietary saturated fatty acids

and cholesterol are decreased to less than or equal to 7% of calories, and less than or equal to 200 mg of cholesterol per day, while our patients received recommendations based on cholesterol intake less than 300 mg/day.

Although the reduction in triglyceride levels was calculated to be 15% (P = 0.125), it was not statistically significant. In the study conducted in 97 males and fimales, who dropped out from dietary weight management after 16-18 weeks of treatment, and after weight loss of 9-9.4 kg, when they rejoin to the program for a second time, the values of serum cholesterol and triglycerides were 15% and 26% less for females, and 17% and 24% less for males, compared to their respective values at the beginning the first attempt (Dhurandhar and Kulkarni, 1995). The above is important as it confirms the need for the longer duration of diet modification, as well as the possibility of longer duration of the effects achieved by diet.

Changes in serum lipid/lipoprotein concentrations after 4 weeks of modified diet and low grade physical activity

| Lipids/lipoproteins | Mean baseline | Std. Error of Mean | Mean after 4 weeks | Std. Error of Mean | Refer-encerange after 4 weeks | t-test | P | %change | Significant |
|---|---|---|---|---|---|---|---|---|---|
| TC (3.1-6.5 mmol/L) | 8.25 | 0.332 | 7.45 | 0.222 | ↓ | 3.772 | 0.001 | -9.7% | + |
| TG (0.11-2.05 mmol/L) | 2.58 | 0.3 | 2.18 | 0.155 | ↓ | 1.607 | 0.125 | -15.5% | - |
| Apo B (0.54-1.47 g/L) | 1.68 | 0.109 | 1.42 | 0.095 | ↓R | 2.811 | 0.011 | -15.5% | + |
| Apo AI (1.08-2.09 g/L) | 1.56 | 0.056 | 1.42 | 0.0551 | ↓R | 3.059 | 0.006 | -9% | + |
| HDL-C (1.06-1.94 mmol/L) | 1.29 | 0.11 | 1.23 | 0.0731 | ↓R | 0.936 | 0.361 | -4.7% | - |
| VLDLC (0.13-1.0 mmol/L) | 1.10 | 0.1 | 0.97 | 0.0697 | ↓R | 1.962 | 0.065 | -11.8% | - |
| LDLC (2.88-4.87 mmol/L) | 5.58 | 0.3 | 5.25 | 0.259 | ↓ | 1.228 | 0.234 | -5.9% | - |
| TC/HDL-C ≤ 5 | 6.84 | 0.556 | 6.57 | 0.503 | ↓ | 0.556 | 0.585 | -3.9% | - |
| Phospholipids 1.81-3.23 mmol/L | 4.18 | 0.169 | 3.75 | 0.0863 | ↓ | 3.236 | 0.004 | -10.3% | + |
| Total lipids (2.7-7.0 g/L) | 8.77 | 0.352 | 7.79 | 0.187 | ↓ | 2.932 | 0.009 | -11.1% | + |
| Alpha lp. (0.25-0.35) | 0.198 | 0.0148 | 0.203 | 0.0153 | ↑ | -0.368 | 0.717 | +2.5% | - |
| Pre $Beta_1$ (0.08-0.22) | 0.24 | 0.0275 | 0.21 | 0.0189 | ↓R | 1.399 | 0.178 | -12.5% | - |
| Beta (0.45-0.48) | 0.56 | 0.0196 | 0.59 | 0.0136 | ↑ | -1.461 | 0.160 | +5.4% | - |
| Index beta/alpha (1.2-2.3) | 4.7 | 0.453 | 4.4 | 0.368 | ↓ | 0.660 | 0.517 | -6.4% | - |
| Degree of lipemia (10-35) | 45 | 1.409 | 40.45 | 1.150 | ↓ | 2.705 | 0.014 | -10.1% | + |
| Atherogenic index (LDL/HDL) (2.4-4.8) | 5.8 | 0.499 | 5.6 | 0.503 | ↓ | 0.556 | 0.585 | -3.4% | - |

*R –values in reference range of laboratory

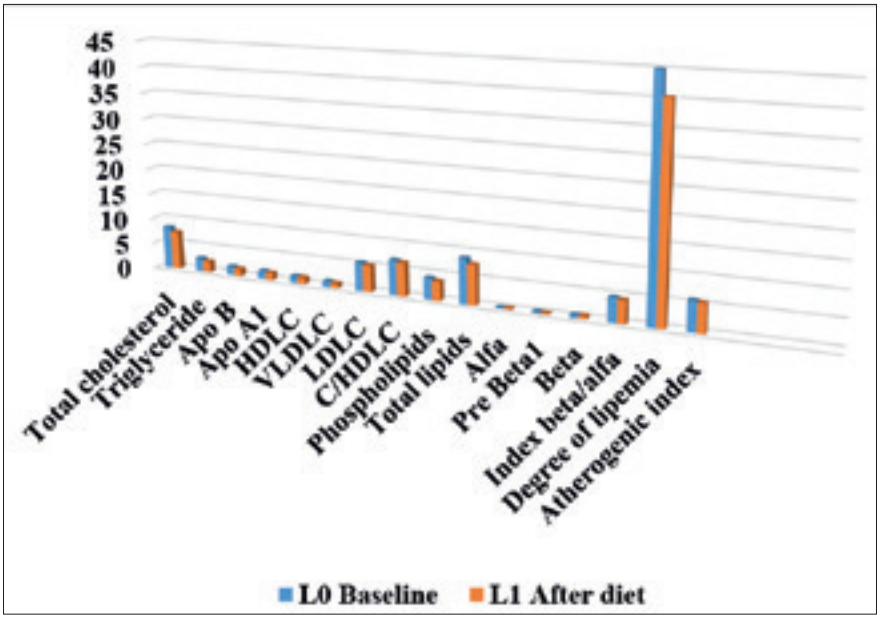


**Chart 1.** Changes in mean values of serum lipids/lipoproteins after 4 weeks of diet and physical activity

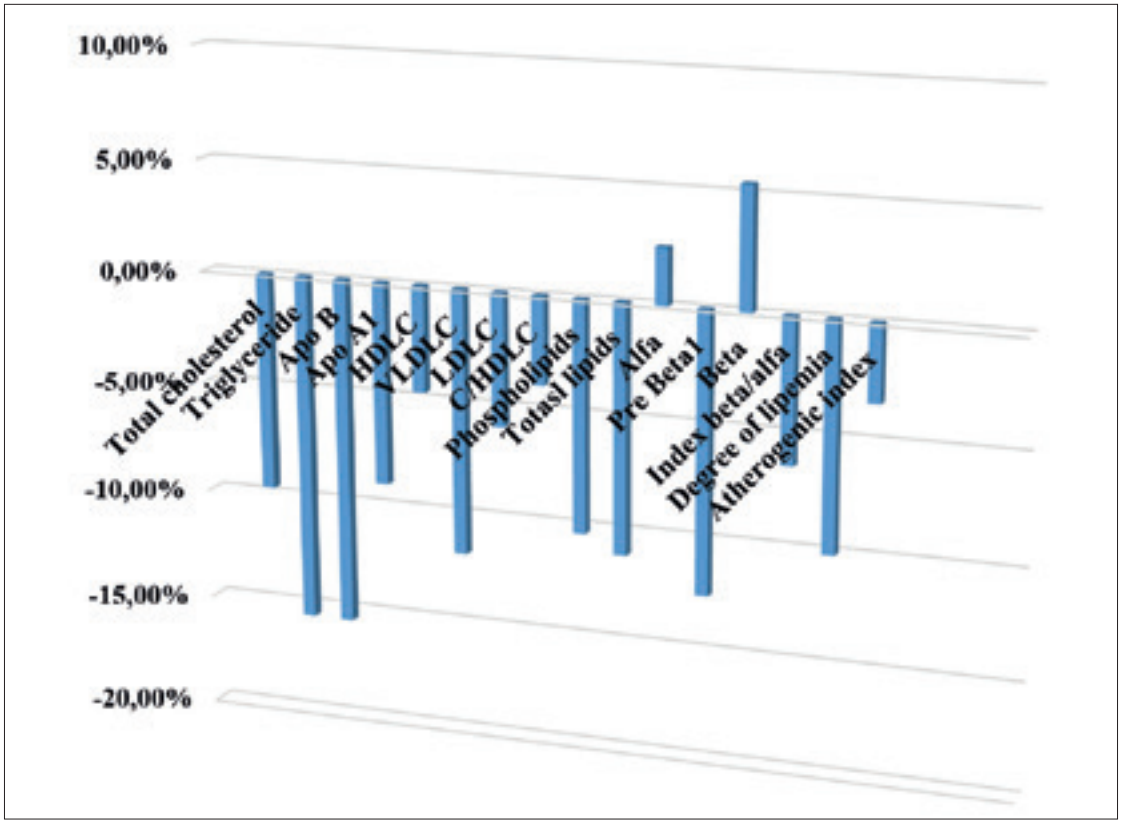


**Chart 2.** Percentual changes of serum lipids/lipoproteins after 4 weeks of diet and physical activity

In our study, a significant decrease in Apo B was recorded, with reduction of Apo B levels of -15.5% (P = 0.011) which was identical to that of triglycerides (–15.5%; P = 0.125), but unlike for triglycerides, this reduction for Apo B was statistically significant. But, if we consider that each VLDL and LDL particle contains one molecule of Apo B and that measuring Apo B accurately measures the total number of VLDL and LDL, of which 90% are LDL (Sniderman, Bergeron and Frohlich, 2001), than, if we add percentages of VLDL and LDL decrease (-11.8% and -5.9%), 90% of this total value is 15.93 what is approximately 15.5%, which in fact is the percent of Apo B reduction.

Analyzing the measurement of the level of Apo AI after period of the diet, a statistically significant decrease of 9% (P=0.006), compared to the baseline values was observed. All values were within a laboratory reference range. This significant decrease of Apo AI did not follow the decrease in HDL of - 4.7% (P = 0.361), which was not significant, but also within a laboratory reference range, although Apo AI is the major structural and functional protein component of HDL and it constitutes approximately 70% of HDL (Mangara, Nanda and Panda, 2016). Similar findings were reported by Lichtenstein and colleagues (1993) after 32-day study phase in which corn oil was replaced with cornoil margarine in stick form, as two thirds of the fat in the National Cholesterol Education Program (NCEP) Step II diet. The change in Step II NCEP diet also resulted in decrease of HDL-C and Apo AI levels, but in reverse manier as mean values for HDL-C decreased by 9% and Apo AI by 0.4% ($P < 0.01$ for HDL-C) on corn oil-enriched diet and 10% and 3% lower on margarine-enriched diet ($P < 0.01$ for HDL-C). These negative effects are attributed to hydrogenation present in margarine (Lichtenstein et al.,1993).

On the other hand, during our study, no statistically significant changes in the level of HDL-C were recorded, which correlates with data from the literature (Solov'eva, Rozhkova, Tvorogova and Kukharchuk, 1999). The percentage changes correlates with the data from literature, so recorded decrease in the level of HDL-C (-4.7%; P = 0.361) is a known negative effect of hypolipemic diets (Knopp et al. 1997, Oliver, 1998).

Our "modified diet" resulted in a non-significant reduction of VLDL-C (-11.8%; P = 0.065), and no significant changes in LDL-C values (- 5.9%; P = 0.234), which is in accordance with data from the literature (Knopp, 1997). However, the reduction achieved for VLDL-C resulted in the value returning to the laboratory reference values, while the LDL-C values all the time were above the upper limit of the laboratory reference values, what is less than 10% as Wadhera and colleagues states in their work from 2016, but after 5 weeks of pure Mediterranean diet.

Our modified diet did not lead to a significant decrease in TC/HDL-C ratio (-3.9%; P = 0.585), but still it was a decrease which is of clinical significance (Mensink, Zock, Kester and Katan, 2003). In his work from 2010, Kelly states that when two versions of a Mediterranean diet were compared with a low-fat diet, the Mediterranean diets lowered the total-to-HDL cholesterol ratio more than the low-fat diet, referring on data from randomized trial that was conducted in asymptomatic persons 55 to 80 years of age at high cardiovascular risk. There were no classification of dyslipidemia, primary or secondary, or even more by Fredrickson classification (Estruchet al., 2006). In this context, our combined "modified diet" (Step I, Step II and Mediterranean), can be considered as a low fat diet.

Taking into account that oxidation of phospholipids, containing polyunsaturated fatty acids present in plasma lipoproteins results in formation of a variety of reactive lipid aldehydes and oxidized phospholipids that convert these lipoproteins to atherogenic particles (Berliner, Leitinger ans Tsimikas, 2009; Linton et al., 2019), the significant decrease of phospholipids in our study (-10.3%; P = 0.004) is considered as an positive effect of our modified diet, as well as for lowering the total lipids concentration that was also statistically significant (-11,1%; P = 0.009).

Total plasma lipid levels in our study were significantly reduced by -11.1% (P = 0.009) what, together with other parameters, serves as a confirmation of adherence to the diet recommended.

As mentioned before, based on localization in relation to serum proteins, in electrophoretic separation lipoproteins are divided into α-lipoprotein, pre-$\beta_1$-lipoprotein, β-lipoprotein, corresponding to HDL, VLDL and LDL. One can assume that increase in α-lipoprotein of +2.5% (P = 0.717), and β-lipoprotein of +5.4% (P = 0.160) recorded in our study are inversly related to HDL or LDLvalues, but it should be kept in mind that values for HDL and LDL are in fact values for HDL-C and LDL-C. Back in 1964, De

Oliveira described in his work that in patients with coronary heart disease, serum β-lipoprotein and its cholesterol content are elevated, whereas levels of serum alpha cholesterol are diminished. Before De Oliveira, Barr and colleagues in 1951, suggested that patients who survived a coronary occlusion (acute myocardial infarction) or with other unequivocal evidence of atherosclerosis-related complications, had several abnormalities in plasma proteins, including a reduction in the alpha lipoprotein content (Ndrepepa, 2021). The baseline values of α-lipoprotein in our study were below lower reference limit (α-lp. = 0.198, reference range 0.25 – 0.35), almost reaching lower reference limit after four weeks of diet (α-lp. = 0.203). For β-lipoprotein, values were all the time above uper reference limit (baseline βlp. = 0.56; after diet βlp. = 0.59). Nevertheless, the increase in α-lipoprotein is a positive effect of our diet while increase in β-lipoprotein still needs to be explained. However, the proatherogenic index beta/alpha decreased by -6.4% (P = 0.517) and all changes were statistically not significant but were the result of adherence to the diet. Further, α-lipoprotein values increased close to the reference ones, pre-$\beta_1$-lipoprotein reached rerefence value after four weeks of diet (pre-$\beta_1$lp. = 0.21, reference values 0.08 - 0.22) and β-lipoprotein values remained above uper reference limit.

The degree of lipemia decreased significantly (-10.1%; P = 0.014) following decrease in triglycerides and lipoproteins which are the most common cause of turbidity (Kroll, 2004). Values of atherogenic index (LDL/HDL cholesterol), as a strong predictor of cardiaovascular events (Barter al, 2007), decreased for -3.4% (P = 0.585) in our study after four weeks of modified diet. Although these values were not statistically significant, the decrease is still evident.

After a period of adherence only to a diet, the subjective feeling characterized by “excellent” was recorded in all 20, or 100% of patients. The loss of body weight ranged from 1.5 to 4 kg, but since the measurement of body weight was not performed every time on the same measuring instrument and under the control of the medical staff, these data were not taken into statistical evaluation.

## CONCLUSION

Recognizing an underestimated value and role of dietary modifications in everyday life of patients with hypercholesterolaemia, we decided to actualize our investigation from 2000, as we witnes that lipid lowering drugs are prescribed almost as soon as elevated serum lipid levels are detected. The reference range values in this study were the values of the Laboratory for Lipids, University Clinical Centre Sarajevo, which are higher than target values recommended by European guidelines. Compared to baseline values, decreased values were recorded for 14 out of 16 laboratory parameters from complete lipidogram. Two parameters, which values increased were antiproatherogenic α-lipoprotein (+2.5%) and proatherogenic β-lipoprotein (+5.4%), both statistically not significant. None of the target values, recommended by European guideline, were achieved. Based on the results from our study, lowering of serum lipid levels was achieved after four weeks of modified diet and low grade of physical activity, with positive effects on lowering serum proatherogenic lipids and on increase of antiproatherogenic α-lipoprotein. Larger number of drug-naïve patients with Type IIa and IIb dyslipoproteinaemia should be included in further investigations, physical activity should be more intense and for a longer duration of modified diet. Our study confirms that modified diet should precede the decision for hypolipemic drug use.

**Limitations**

*Comparisons with results from other studies were intentionally done based on data from late 1990s and early 2000s, as our study was conducted during that period and target values for serum lipids were published in 1998 by American and European guidelines for the management of dyslipidaemias. In recent literature, the results of different statin drugs efficacy are presented, while there is a lack of data on the effectiveness of the diet itself.*

**Conflict of interest**
*There are no conflicts of interest.*
**Financial support and sponsorship:** *None*

# REFERENCES

Barter, P., Gotto, A.M., LaRosa, J.C., Maroni, J., Szarek, M., Grundy, S.M., Kastelein, J.J., Bittner, V., & Fruchart, J.C. (2007). Treating to New Targets Investigators. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med, 27;357(13):1301-10. doi: 10.1056/NEJMoa064278. PMID: 17898099.

Beaumont, J.L., Carlson, L.A., Cooper, G.R., Fejfar. Z., Fredrickson, D.S., & Strasser, T. (1970). Classification of hyperlipidaemias and hyperlipoproteinaemias. Bull World Health Organ, 43(6):891-915. PMID: 4930042; PMCID: PMC2427808.

Berliner, J.A., Leitinger, N., & Tsimikas, S. (2009). The role of oxidized phospholipids in atherosclerosis. J Lipid Res, 50 Suppl(Suppl):S207-12. doi: 10.1194/jlr.R800074-JLR200. Epub 2008 Dec 4. PMID: 19059906; PMCID: PMC2674746.

Chandra, K.S., Bansal, M., Nair, T., Iyengar, S.S., Gupta, R., Manchanda, S.C., Mohanan, P.P., Rao, V.D., Manjunath, C.N., Sawhney, J.P., Sinha, N., Pancholia, A.K., Mishra, S., Kasliwal, R.R., Kumar, S., Krishnan, U., Kalra, S., Misra, A., Shrivastava, U., & Gulati, S. (2014). Consensus statement on management of dyslipidemia in Indian subjects. Indian Heart J, 66 Suppl 3(Suppl 3): S1-51. doi: 10.1016/j.ihj.2014.12.001. Epub 2014 Dec 24. PMID: 25595144; PMCID: PMC4297876.

De Oliveira, J.M. (1964). Changes in the levels of serum cholesterol and beta lipoprotein according to age, sex, and the existence of coronary heart disease. Am Heart J, 67:177-83. doi: 10.1016/0002-8703(64)90367-9.Available at: https://www.sciencedirect.com/science/article/abs/pii/0002870364903679 [Accessed: 27 December, 2023]

Dhurandhar, N.V., & Kulkarni, P.R. (1995). Serum cholesterol and triglyceride levels in weight reduction program dropouts. Int J Food Sci Nutr, 46(1):17-20. doi: 10.3109/09637489509003381. PMID: 7712338.

Diab, A., Dastmalchi, L.N., Gulati, M., & Michos ED. (2023). A Heart-Healthy Diet for Cardiovascular Disease Prevention: Where Are We Now? Vasc Health Risk Manag. 19:237-253. doi: 10.2147/VHRM.S379874. PMID: 37113563; PMCID: PMC10128075.

Dwyer, J.T. & Roy, J. (1997). Dijetoterapija in Harrison Principi interne medicine, Trinaesto izdanje, Izdavačka kuća Placebo d.o.o. Split, 400-407

Farnier, M., & Davignon, J. (1998). Current and future treatment of hyperlipidemia: the role of statins. American Journal of Cardiology, 82:3J-10J

Jansen, S., Lopez-Miranda, J., Salas, J., Castro, P., Paniagua, A.J., Lopez-Segura, F., Ordovas, M.J., Jimenez-Pereperez, A.J., Blanco, A., & Perez-Jimenez, F. (1998). Plasma Lipid Response to Hypolipidemic Diets in Young Healthy Non-Obese Men Varies with Body Mass Index. The Journal of Nutrition, 128(7):1144-1149

Kelly, R.B. (2010). Diet and exercise in the management of hyperlipidemia. Am Fam Physician, 81(9):1097-102. PMID: 20433126.

Knopp, R.H., Walden, C.E., Retzlaff, B.M., McCann, B.S., Dowdy, A.A., Albers, J.J., Gey, G..O, & Cooper, M.N. (1997). Long-term cholesterol-lowering effects of 4 fat-restricted diets in hypercholesterolemic and combined hyperlipidemic men. The Dietary Alternatives Study. JAMA, 278(18):1509-15. PMID: 9363971.

Kris-Etherton, P.M., Krummel, D., Russell, M.E., Dreon, D., Mackey, S., Borchers, J., & Wood, P.D. (1988). The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. J Am Diet Assoc, 88(11):1373-400. PMID: 2846672.

Kroll, M. (2004). Evaluating interference caused by lipaemia. *Clin Chem.* 2004;50:1968–1969.

in Castro-Castro MJ, Candás-Estébanez B, Esteban-Salán M, Calmarza P, Arrobas-Velilla T, Romero-Román C, Pocoví-Mieras M, Aguilar-Doreste JÁ; Commission on Lipoprotein and Vascular Diseases, Sociedad Española de Química Clínica. Removing Lipemia in Serum/Plasma Samples: A Multicenter Study. Ann Lab Med, 2018; 38(6):518-523. doi: 10.3343/alm.2018.38.6.518. PMID: 30027694; PMCID: PMC6056396.

Lichtenstein, A.H,, Ausman, L.M., Carrasco, W., Jenner, J.L., Ordovas, J.M., & Schaefer, E.J. (1993). Hydrogenation impairs the hypolipidemic effect of corn oil in humans. Hydrogenation, trans fatty acids, and plasma lipids. Arterioscler Thromb, 13(2):154-61. doi: 10.1161/01.atv.13.2.154. PMID: 8427852.

Linton, M.R.F., Yancey, P.G., Davies, S.S., et al. (2019). The Role of Lipids and Lipoproteins in Atherosclerosis. [Updated 2019 Jan 3]. In: Feingold, K.R., Anawalt, B., Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK343489/,

Lorgeril, M. et al. (1999). Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction: Final report of the Lyon Diet Heart Study. Circulation, 99:779

Mann, S., Beedie, C., & Jimenez A. (2014). Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendations. Sports Med. 44(2):211-21. doi: 10.1007/s40279-013-0110-5. PMID: 24174305; PMCID: PMC3906547.

Mensink, R.P., Zock, P.L., Kester, A.D., & Katan, M.B. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr, 77(5):1146-55. doi: 10.1093/ajcn/77.5.1146. PMID: 12716665.

Mortensen, M.B., Dzaye, O., Bøtker, H.E., Jensen, J.M., Maeng, M., Bentzon, J.F., Kanstrup, H., Sørensen, H.T., Leipsic, J., Blankstein, R., Nasir, K., Blaha, M.J., & Nørgaard, B.L. (2023). Low-Density Lipoprotein Cholesterol Is Predominantly Associated With Athero-

sclerotic Cardiovascular Disease Events in Patients With Evidence of Coronary Atherosclerosis: The Western Denmark Heart Registry. Circulation. 147(14):1053-1063. doi: 10.1161/CIRCULATIONAHA.122.061010. Epub 2023 Jan 9. PMID: 36621817; PMCID: PMC10073288.

National Cholesterol Education Program - NCEP. (1994). Second Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). Circulation, 89(3):1333-445. doi: 10.1161/01.cir.89.3.1333. PMID: 8124825.

Ndrepepa, G. (2021) High-density lipoprotein: a double-edged sword in cardiovascular physiology and pathophysiology. ReviewArticle.J Lab Precis Med, 6:28: 1-24 | https://dx.doi.org/10.21037/jlpm-21-32

Oliver, M.F. (1998). Cholesterol lowering diets and coronary heart disease. Letters BMJ, 317:1253

Pescatello, L.S., Murphy, D., & Costanzo D. (2000). Low-intensity physical activity benefits blood lipids and lipoproteins in older adults living at home. Age Ageing. 29(5):433-9. doi: 10.1093/ageing/29.5.433. PMID: 11108416.

Sloop, G.D. (1999). A critical analysis of the role of cholesterol in atherogenesis. Atherosclerosis. 142(2):265-8. doi: 10.1016/s0021-9150(98)00270-6. PMID: 10030376.

Sniderman, A.D., Bergeron, J., & Frohlich, J. (2001). Apolipoprotein B versus lipoprotein lipids: vital lessons from the AFCAPS/TexCAPS trial. CMAJ, 164(1):44-7. PMID: 11209748; PMCID: PMC80634.

Solov'eva, E.I., Rozhkova, T.A., Tvorogova, M.G., & Kukharchuk, V.V. (1999). Cerivastatin-a new synthetic 3-hydroxy-3-methylglutaryl (HMG) inhibitor: effect of 0,2 mg dose in patients with primary hyperlipidemias. Ter Arkh, 1999;71(8):30-4

Steinberg, D., Gotto, A.M. (1999). Preventing Coronary Artery Disease by Lowering Cholesterol Levels – Fifty Years From Bench to Bedside. JAMA 282: 2043-2050

Tang, J.L., Armitage, J.M., Lancaster, T., Silagy, C.A., Fowler, G.H., Neil, H.A. (1998). Systematic review of dietary intervention trials to lower blood total cholesterol in free-living subjects. BMJ, 18;316(7139):1213-20. doi: 10.1136/bmj.316.7139.1213. PMID: 9552999; PMCID: PMC28525.

Wadhera, R.K., Steen, D.L., Khan, I., Giugliano, R.P., & Foody, J.M. (2016). A review of low-density lipoprotein cholesterol, treatment strategies, and its impact on cardiovascular disease morbidity and mortality. J Clin Lipidol. 10(3):472-89. doi: 10.1016/j.jacl.2015.11.010. Epub 2015 Nov 25. PMID: 27206934.

Williams, P.T., & Thompson, P.D. (2013). Walking versus running for hypertension, cholesterol, and diabetes mellitus risk reduction. Arterioscler Thromb Vasc Biol. 33(5):1085-91. doi: 10.1161/ATVBAHA.112.300878. Epub 2013 Apr 4. PMID: 23559628; PMCID: PMC4067492.

Wood, D., De Backer, G., Faergeman, O., Graham, I., Mancia, G., & Pyörälä, K. (1998). Prevention of coronary heart disease in clinical practice. Recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention. European Society of Cardiology. European Atherosclerosis Society. European Society of Hypertension. International Society of Behavioural Medicine. European Society of General Practice/Family Medicine. European Heart Network. Eur Heart J, 19(10): 1434-1503

World Health Organization (2020) The top 10 causes of death. 9 December 2020. Available at: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death [Accessed: 14 December, 2023]

World Health Organization/Fredrickson classification of primary hyperlipidemias. Last reviewed 21 Jul 2021. Available at: https://gpnotebook.com/pages/cardiovascular-medicine/whofredrickson-classification-of-primary-hyperlipidaemias [Accessed: 14 December, 2023]

Zmysłowski, A., & Szterk A. (2017). Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols. Lipids Health Dis. 2;16(1):188. doi: 10.1186/s12944-017-0579-2. PMID: 28969682; PMCID: PMC5625595.

*Recived: December 29, 2023*
*Accepted: January 20, 2024*