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PCSK9 inhibitors - impact on lipid profile and cardiovascular outcome after a 4-year follow-up

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PCSK9 Inhibitors – impact on lipid profile and cardiovascular outcome after a 4-year follow-up

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Table of contents

AGRADECIMENTOS……..4
LIST OF ABBREVIATIONS……7
ABSTRACT……8
RESUMO……10
INTRODUCTION……12
MATERIALS AND METHODS……14
RESULTS……19
BASIC LIPID PROFILE……19
GROUP ANALYSIS……22
SECONDARY ENDPOINT……22
ADDITIONAL ANALYSIS.....22
SAFETY……22
DISCUSSION.....24
CONCLUSION.....30
REFERENCES.....31
SUPPLEMENTARY APPENDIX.....35

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List of Abbreviations

AEA – Adverse Events Analysis

ApoA1 – Apolipoprotein A1

ApoB – Apolipoprotein B

CHUC – Centro Hospitalar e Universitário de Coimbra

CV - Cardiovascular

CVD – Cardiovascular Diseases

EMA - European Medicines Agency

HDL – High-Density Lipoprotein

HDL-C – High-Density Lipoprotein

FDA – U.S. Food and Drug Administration

LDL – Low-Density Lipoprotein

LDL-C – Low-Density Lipoprotein Cholesterol

LPA – Lipid Profile Analysis

Lp(a) – Lipoprotein (a)

nAbs – Neutralizing antibodies

PCSK9 - Proprotein convertase subtilisin/kexin type 9

RCT – Randomized Control Trials

SAE – Serious Adverse Event

Abstract

Background:

As cardiovascular (CV) diseases continue to be the leading cause of mortality in developed countries[1–3], being dyslipidemia one of the most important risk factors [1,2], major efforts have been made to control patients' lipid profile, particularly LDL-C[2]. PCSK9 inhibitors have been developed and initial efficacy and safety results have been promising, indicating decreases of around 60% in LDL-C levels with no safety issues[1,4]. However, the most important studies have short follow-up periods[5–7], thereby the need of performing longer studies in order to understand whether time alters the effects of this new class of drugs. The primary goal of this work is to analyze long-term safety and efficacy of PCSK9 inhibitors.

Material/Methods:

A total of 116 patients (79.3% men, mean age of 67.5 years) received Evolocumab subcutaneously (140mg every two weeks or 420mg monthly) at CHUC, after having finished the FOURIER study (median 2.2 years) and were followed-up during 96 weeks with squeduled visits every 24 weeks and an additional visit in week 12. In those prespecified visits, we assessed the patients' lipid profile data and gathered information about every adverse event. Afterwards, we appraised the evolution of the lipid profile during the 96-week follow-up and analyzed the incidence of adverse events and major CV events (defined as: CV death, myocardial infarction, stroke, hospitalization due to unstable angina and coronary revascularization). Events possibly associated with extremely-low LDL-C values were taken into consideration.

Results:

We found an improvement in lipid profile across every time point, in almost every major measure, including a 65% decrease in LDL-C levels ($p<0.001$). Lipoprotein (a) levels did not decrease with statistical significance ($p=0.053$). Major CV events occurred in 5.2% of patients. The long-term exposure to these drugs does not seem to cause any adverse event. We found no adverse event related to extremely-low LDL-C levels and observed a diminished decrease in LDL-C values in active smokers ($p=0.005$).

Conclusion:

PCSK9 inhibitors keep collecting great evidence of its beneficial effects. Trough time, its improvement on lipid profile did not diminish and kept on showing a significant decrease of major CV events, while leading to no increase in incidence of adverse events.

Key words: PCSK9 inhibitors; Dyslipidemia; Cardiovascular risk; LDL-C; Lipoprotein (a).

Resumo

Introdução:

As doenças cardiovasculares continuam a ser a principal causa de morte em países desenvolvidos[1–3], e a dislipidémia um dos principais fatores de risco cardiovasculares[1,2]. Têm-se envidado esforços para controlar o perfil lipídico dos doentes, com especial ênfase nos níveis de LDL-C[2]. Os inibidores da PCSK9 têm vindo a ser desenvolvidos e os resultados iniciais têm sido promissores, indicando decréscimos dos níveis de LDL-C na ordem dos 60% e não levantando preocupações relativamente à segurança dos mesmos[1,4]. Não obstante, devido aos curtos tempos de seguimento dos estudos mais importantes[5–7], há necessidade de realizar estudos com o intuito de descobrir se o tempo altera os efeitos desta nova classe de fármacos. O objetivo principal deste trabalho é avaliar a eficácia e segurança dos inibidores da PCSK9 a longo prazo.

Materiais e Métodos:

Um total de 116 doentes (79.3% homens, idade média de 67.5 anos) receberam Evolocumab subcutâneo (140mg a cada duas semanas ou 420mg mensalmente) no CHUC após terminarem o estudo FOURIER (tempo de seguimento mediano de 2.2 anos) e foram seguidos durante 96 semanas com visitas agendadas a cada 24 semanas e uma visita adicional às 12 semanas. Nessas visitas programadas, obtivemos o perfil lipídico dos doentes e recolhemos informações relativas aos eventos adversos. Avaliámos a evolução do perfil lipídico durante as 96 semanas e analisamos a incidência de eventos adversos e de eventos cardiovasculares definidos como major (morte por causa cardiovascular, enfarte agudo do miocárdio, AVC, internamento por angina instável e revascularização coronária). Foram tidos em conta eventos possivelmente associados a níveis extremamente baixos de LDL-C.

Resultados:

Observámos melhoria em quase todos os parâmetros lipídicos importantes durante todo o tempo de seguimento, incluindo um decréscimo de 65% nos valores de LDL-C ($p<0.001$). Os níveis de Lipoproteína (a) não diminuíram de forma estatisticamente significativa ($p=0.053$). Ocorreram eventos cardiovasculares major em 5.2% dos pacientes. A exposição

prolongada a este fármaco parece ser segura, não tendo ocorrido nenhum evento adverso na nossa amostra. Não foram identificados eventos adversos relacionados com níveis extremamente baixos de LDL-C. Os fumadores ativos parecem ter uma resposta menos positiva no que toca ao decréscimo dos valores de LDL-C (p=0.005).

Conclusão:

Os inibidores da PCSK9 continuam a mostrar boas evidências dos seus efeitos benéficos nos doentes que recorrem a esta medicação; mantendo os seus efeitos inalterados com o tempo, diminuindo a incidência de eventos cardiovasculares major e sem levar a aumento na incidência de eventos adversos.

Palavras-chave: Inibidores da PCSK9; Dislipidémia; Risco cardiovascular; LDL-C; Lipoproteína (a).

Introduction

Cardiovascular diseases (CVD) are still the leading cause of mortality and morbidity in Portugal and other developed countries[1–3] killing over four million people every year, just in Europe[2]. One of the most important Cardiovascular (CV) risk factors is LDL-C levels. It has been shown that CV risk decreases with the reduction of LDL-C levels. Moreover, it keeps decreasing as lower levels of LDL-C are achieved, regardless of the approach[1–3,8–10]. Even though statins continue to be the first line of treatment, as recommended by the newest guidelines[2], other molecules have been studied and approved for clinical use. Among those, PCSK9 inhibitors have shown great promise.

PCSK9 is an important protein in the lipid (and more specifically LDL-C) metabolism. It acts by binding itself to the EGF-like repeat homology domain-A (EGF-A) of the hepatic LDL receptor (LDLR). This bind decreases the amount of LDLR on the surface of the hepatocyte by inhibiting its recycling. As LDLR is responsible for the removal of LDL-C from circulation, it's easy to understand why it is so appealing to target this pathway for lipid-lowering therapy. The structure and function of the PSCK9 protein are described, in detail, by multiple authors[4,11,12].

Currently, there are two fully human monoclonal antibodies, acting as PCSK9 inhibitors, approved for clinical use. Two major trials studied the effect and safety of these new drugs, the ODYSSEY trial[5], focused on Alirocumab and the FOURIER trial[6], focused on Evolocumab; both provided encouraging results. In fact, not only have they had a significant reduction of major CV events and reduced LDL-C by approximately 60%, but also had great safety outcomes, presenting little to no major adverse events[4–6].

A recent meta-analysis, which included 28 randomized control trials (RCT's) (including ODYSSEY and FOURIER), described similar results[1].

One advantage of PSCK9 inhibitors compared to statins is their effect on Lipoprotein (a) [Lp(a)]. The association between Lp(a) and higher coronary risk is well documented[2,8,13–15] as it is the inability of statins to consistently reduce its levels[2,8,14,15]. In fact, lowering Lp(a) is still a challenge for clinicals. The FOURIER trial presented a reduction of 27% in Lp(a) levels[6], which are promising results for solving this problem.

Even though Evolocumab and Alirocumab presented promising results, none of the drugs had a significant effect on lowering CV mortality. This may be explained by the short follow-up time of both trials[7] (median of 2.2 years for FOURIER and 2.8 years for ODYSSEY),

which is their biggest limitation, thus the need to continue to follow these patients, in order to understand the long-term effect of PCSK9 inhibitors. Our work has, precisely, that objective: to study the long-term effect of PCSK9 inhibitors; particularly, to study if their effect on lipid profile and their safety keep on suggesting that, sooner or later, PCSK9 inhibitors will have a crucial role in the approach to dyslipidemia.

Materials and Methods

Sample's description

This study included 116 patients that had participated in the FOURIER trial.

The FOURIER trial was a randomized, double-blind, placebo-controlled, multinational clinical trial[6]. Inclusion criteria to this trial were: patient's age between 40 and 85 years at the date of their inclusion and clinical evidence of atherosclerotic cardiovascular disease defined as: history of myocardial infarction, non-hemorrhagic stroke, or symptomatic peripheral artery disease - or additional criteria to be considered at higher cardiovascular risk. Moreover, patients' fasting LDL-C level should be, at least, 70 mg per deciliter (1.8 mmol per liter) or a non-HDL cholesterol level of, at least, 100 mg per deciliter (2.6 mmol per liter), while taking an optimized lipid-lowering regimen, preferably a high intensity statin (minimum of 20mg atorvastatin daily or equivalent).

Evolocumab was taken subcutaneously at a dose of 140mg every two weeks or, alternatively, in a unique monthly dose of 420mg.

During the FOURIER trial, there were 160 patients followed at Centro Hospitalar e Universitário de Coimbra (CHUC). Afterward, 39 patients decided not to keep taking the medication while being followed at our center. The reasons for their decision could not be obtained. Furthermore, out of the 121 remaining patients, five missed one or more periodic visits and, therefore, were excluded due to missing data.

The remaining 116 patients that comprised the study sample fulfilled the inclusion criteria mentioned above, completed the FOURIER trial and accepted continuing Evolocumab while being followed in CHUC.

One patient died early in the trial, between week 24 and week 48, due to chronic heart failure; there was no coronary event reported during his follow-up. This was considered in the analysis of adverse events, even though this patient's lipidic data haven't been considered due to the design of the paired analysis during the 96 weeks. Therefore, 116 patients were analyzed for adverse events but only 115 were considered for lipid data variations (Figure 1).

Figure 1. Algorithm for global study sample.

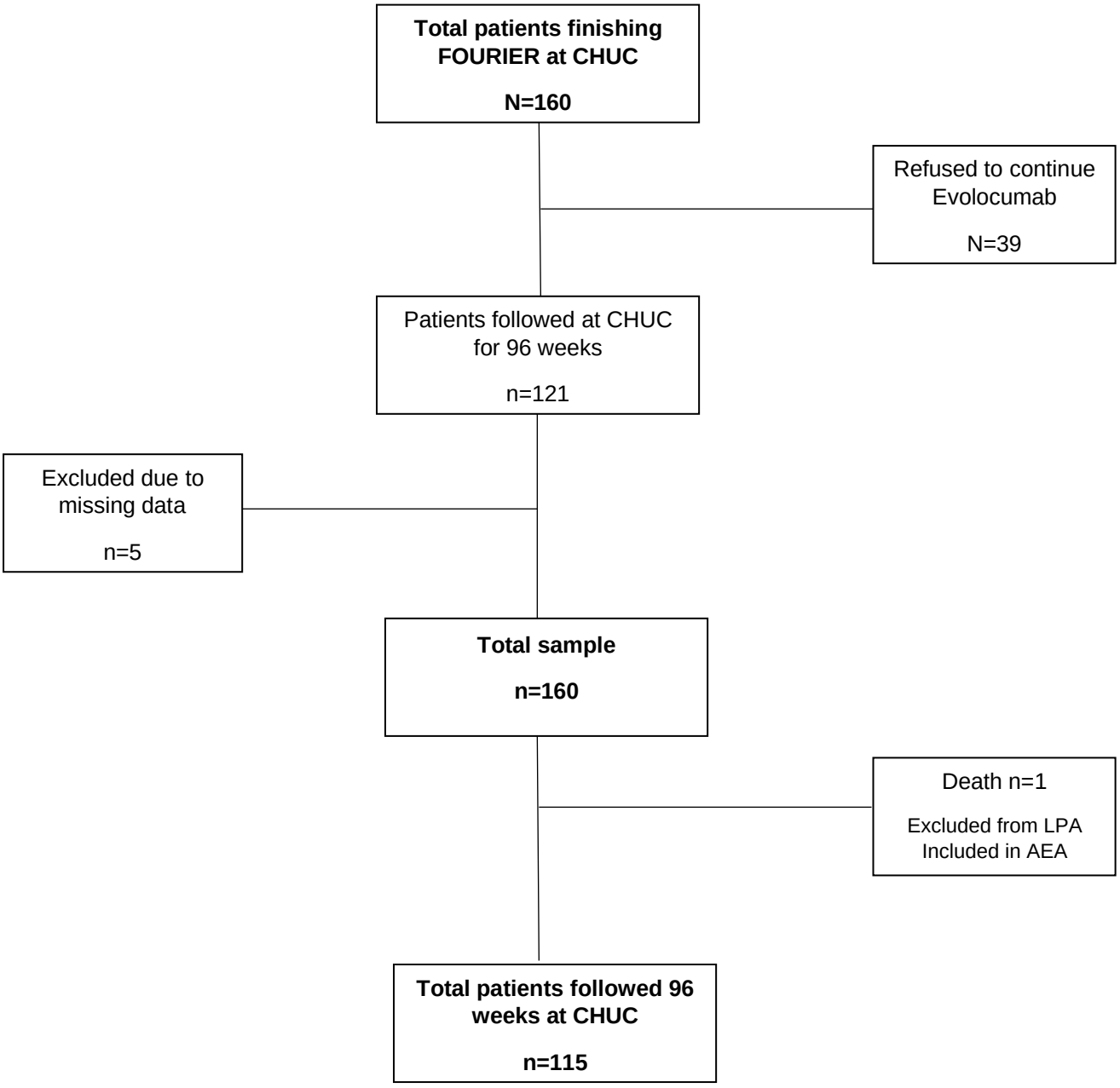

Figure 1. Flow-chart for selecting study sample. From the initial 160 patients of the FOURIER study, 39 refused to continue Evolocumab, five presented missing data, thus 116 patients were included in this study. One patient died, thereby, only 115 patients presented complete data for the 96 weeks follow-up lipidic study.

AEA – Adverse events analysis.

LPA – Lipid profile analysis

Sample's age, at the beginning of our study (June 2017), ranged between 44 and 87 years old (67,5 +/- 9.53) and 79.3% were men. Relevant comorbidities found included diabetes (36.2%); concerning daily habits, 4.3% were active smokers. Additional information about the sample can be found in Table 1.

Table 1. Baseline Characteristics of the 116 patient that participated in the study.

Characteristic	n=116
Age – years	67.5 +/- 9.5
Male sex – no. (%)	92 (79.3%)
BMI – kg/m2	28,8 +/- 4.4
Urban Residents – no. (%)	29 (25%)
Cardiovascular Risk Factors – no. (%)	
Diabetes	42 (36.2%)
Hypertension	114 (98.3%)
Active smoker	5 (4.3%)
Statin use – no. (%)	
High intensity	108 (93.1%)
Moderate intensity	6 (5.2%)
Unknown	1 (0.9%)
Previous history	
Myocardial Infarction	99 (85.3%)
Stroke	19 (16.4%)
Percutaneous coronary intervention	79 (68.1%)
Lipid measures	
Median LDL cholesterol – mmol/L (IQR)	2.23 (1.92-2.75)
Median Triglycerides – mmol/L (IQR)	1.25 (0.86-1.85)
Mean HDL cholesterol – mmol/L (95% CI)	1.28 (+/- 0.39)
Median Lipoprotein (a) – nmol/L (IQR)	60 (18-183)
Median ApoB – mmol/L (IQR)	0.82 (0.72-0.95)
Median ApoA1 – mmol/L (IQR)	1.36 (1.23-1.53)

Characteristics are shown in absolute number and percentage of patients that have the characteristic in question, with the exception of lipid measures, which are shown in mmol/L or nmol/L.

Data collecting and analyses

Lipid data were collected from blood samples in prespecified visits at 12, 24, 48, 72 and 96 weeks of follow-up. At each time point, 5ml of blood was drawn, requiring a fasting period greater than nine hours. Blood samples were, then, frozen within one hour. Later, that sample was shipped to the laboratory where they would be analyzed. To collect more precise results, LDL-C levels below 1mmol/L were recalculated using ultracentrifugation. To calculate the remaining measures, it was used regular laboratory techniques.

Baseline levels were collected from the FOURIER trial thus, they date back to 2013.

Adverse events were reported and collected in the same periodic visits. Reports from any medical care needed during the time of follow-up were available to our team and are included in this study. We divided the population into two pairs of groups, with patients above and below LDL-C levels of 1mmol/L and 0.4mmol/L and analyzed their differences.

We also analyzed how active smocking and diabetes affected the efficacy of PCSK9 inhibitors in improving lipid profile.

Endpoints

In this study, the primary endpoint was to analyze the long-term effects of Evolocumab on lipid profile, along with its safety.

As a secondary endpoint, we tried to analyze the practical benefit of Evolocumab on its users. In order to achieve that, we used a composite of major CV events and analyzed how many patients have experienced any of those events. This composite of major CV events was based on the primary endpoint of the FOURIER trial. Therefore, we considered as major the following events: cardiovascular death, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, unstable angina requiring hospitalization or coronary revascularization. For a patient to be included in the said composite, he needed the occurrence of just one of the events mentioned. Patients continued to be followed after the occurrence of any event.

A secondary analysis was performed in order to evaluate variability along the 96 weeks of follow-up in clinical data obtained

Statistical analysis

Data were analyzed in SPSS™ (version 25.0 for Windows) and was interpreted at a 5% significance level.

To analyze lipid data, we used the Shapiro-Wilk test for normality assessment previously to assessing other repeated measures ANOVA assumptions, such as sphericity. Whenever it was possible, we applied that method; otherwise, the Friedman test was applied. To compare clinical data between diabetic patients and active smokers, a Mann-Whitney Utest for independent samples was applied.

Categorical data were evaluated using the Chi-square test or the Fisher exact test according to the Cochran’s rules, To compare the two groups for adverse events we used an exact

Fisher test, comparing the incidence of neurocognitive function affection, worsening of control of Diabetes Mellitus, Myocardial infarction, hospitalization due to Unstable angina and serious adverse events. We used the same test to analyze the incidence of the secondary endpoint.

Results

The study sample considered 116 patients, which had concluded the FOURIER trial and agreed to continue Evolocumab while being followed at Centro Hospitalar e Universitário de Coimbra. These patients were followed for 1.8 years (from July 2017 to May 2019) additionally to the median of 2.2 years (interquartile range [IQR] of 1.8-2.5) from the FOURIER trial, adding, to a total of, approximately, four years.

Baseline characteristics are described in Table 1.

Basic lipid profile

At baseline, the median LDL-C level was of 2.23mmol/L (interquartile range, 1,92 to 2,75). At week 12 (2.4 years of follow-up), we found a statistical relevant difference, reducing from 1.58mmol/L (0.65-2.51; IQR) ($p<0.001$) to a median level of 0.73mmol/L (IQ, 0.41; 1.04), leading to a median absolute decrease of 71%. At week 96 (4 years of follow-up), median LDL-C levels were 0.83mmol/L (IQR, 0.57; 1.37). We found a statistically significant difference in LDL-C levels when comparing baseline data to week 96 data ($p<0.001$), with a median absolute decrease of 1.45mmol/L (0.57-2.33; IQR) or 65% (25.6-104.5; IQR). We also found a median increase between week 12 and week 96 of 15.1% ($p=0.001$). Further details can be found in Table 2 and Supplementary Appendix I.

Lp(a) levels also presented statistically significant variations; presenting a reduction of its levels; baseline median Lp(a) level was 60nmol/L at the study beginning (IQR; 18-183) and 46nmol/L at week 12 (IQR, 10-156), leading to a decrease of 18.3% ($p<0.001$). At week 96, median levels were 44nmol/L (IQR; 20-161). We found no statistically significant difference between baseline levels and W96 ($p=0.053$). Despite this, we found a statistical relevant median difference (reduction of 13.3%) between baseline levels and levels at week 24 ($p=0.004$) or week 72 ($p=0.001$). There were also statistically significant differences (increasing) between week 12 and all subsequent time points ($p<0.001$ for every pair). Further details can be found in Table 3 and Supplementary Appendix I.

Table 2. Median LDL-C levels (mmol/L) across all six time points of the study.

LDL-C	Med [Q1; Q3] (mmol/L)	p-value vs Baseline
Rand	2,23 [1,92; 2,75]	
W12	0,73 [0,41; 1,04]	**<0.001**
W24	0,70 [0,41; 1,14]	**<0.001**
W48	0,67 [0,46; 1,18]	**<0.001**
W72	0,67 [0,44; 1,27]	**<0.001**
W96	0,83 [0,57; 1,37]	**<0.001**

P-values show a comparison between the said value and Baseline levels. We found one other statistically relevant variation:
W12 vs W96 p= 0.001
There were no other statistically relevant oscillations

Table 3. Median Lp (a) levels (mmol/L) across all six time points of the study.

Lp(a)	Med [Q1; Q3] (nmol/L)	p-value vs Baseline
Rand	60,00 [18,00; 183,00]	
W12	46,00 [10,00; 156,00]	**<0.001**
W24	42,00 [17,00; 171,00]	**0.004**
W48	48,00 [19,00; 157,00]	0.103
W72	41,00 [17,00; 168,00]	**0.001**
W96	44,00 [20,00; 161,00]	0.053

P-values show a comparison between the said value and Baseline levels. We found other statistically relevant variations:
W12 vs W24; W12 vs W48; W12 vs W72; W12 vs W96; p<0.001 for all comparisons
There were no other statistically relevant oscillations

ApoB and Triglycerides also presented statistically significant differences between baseline and week 96, where we could observe a reduction of the mean and or/median values; apoA1 and HDL-C also presented statistically significant differences during the same time (p<0.001 for every pair compared), but in the other direction, increasing mean and/or median values. Further details can be found in Supplementary Appendix I.

Figures 1 and 2 show the evolution of the six measures studied and referenced before.

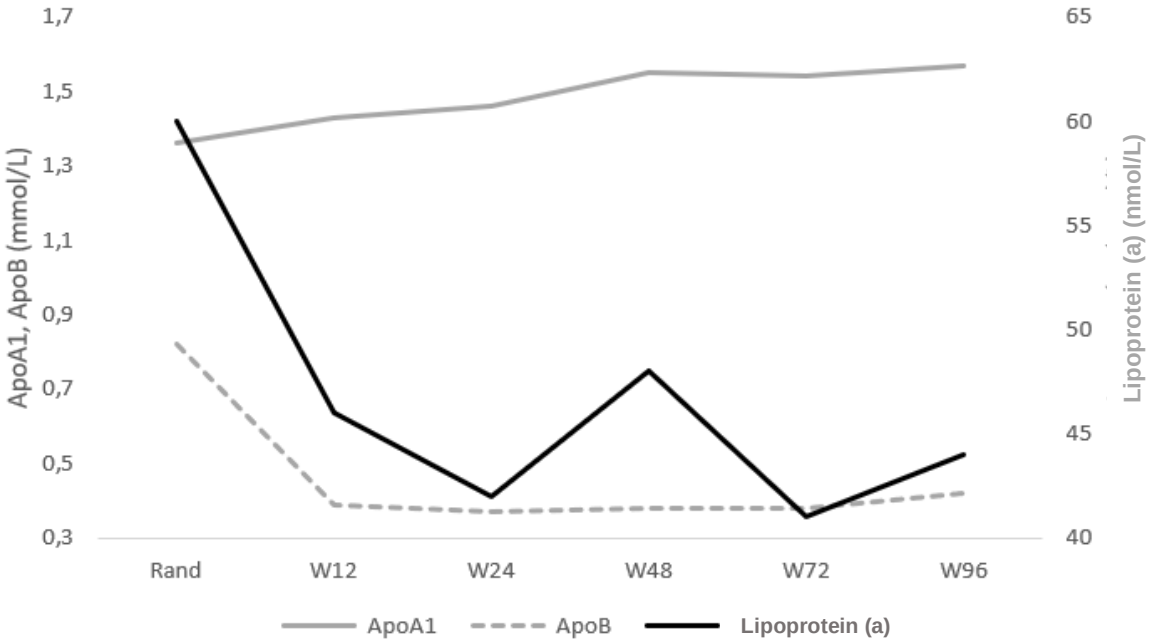

Figure 1. Oscillations in apoA1 (apolipoprotein A1), ApoB (apolipoprotein B) in mmol/L and Lp(a) [Lipoprotein (a)] in nmol/L, across all time points.

Rand (Baseline values), W12 (median of 2.4 years after Baseline), W24 (median of 2.7 years after Baseline), W48 (median of 3.1 years after Baseline), W72 (median of 3.6 years after Baseline) and W96 (median of 4 years after Baseline). For further details see Supplementary Appendix I.

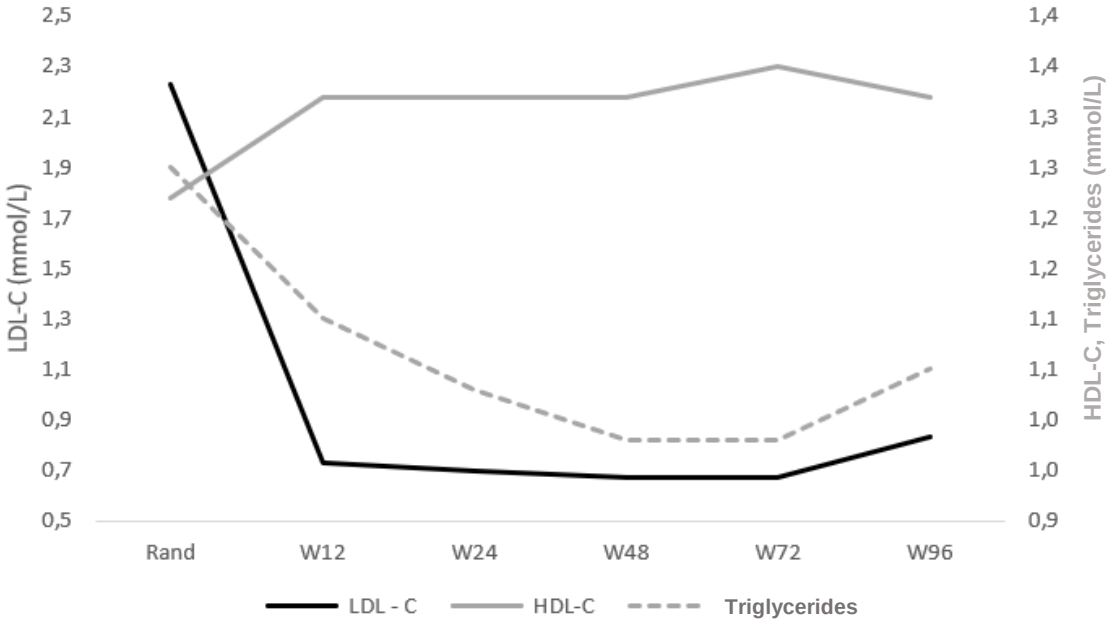

Figure 2. Oscillations in LDL-C, HDL-C and Triglycerides in mmol/L across all timepoints. Rand (Baseline values), W12 (median of 2.4 years after Baseline), W24 (median of 2.7 years after Baseline), W48 (median of 3.1 years after Baseline), W72 (median of 3.6 years after Baseline) and W96 (median of 4 years after Baseline). For further details see Supplementary Appendix I.

Group analysis

We also analyzed the impact active of smoking and diabetes in the efficacy of Evolocumab. If by one hand, we found no significant differences between diabetic and non-diabetic patients, we also established an effect of active smoking and less satisfying results in lowering apoB (p=0.006) and LDL-C (p=0.005) levels.

Secondary endpoint

In our study, we observed an occurrence of 5.2% (6 patients) of the composite of major CV events, defined as a secondary endpoint, during the 1.8-year follow-up.

Additional analysis

When comparing patients with LDL-C levels above and below 1mmol/L, we found that people which presented LDL-C levels above that cut point reported composite of major CV events more frequently than the others (p=0.043). The same association was found for myocardial infarction (p=0.027).

Safety

We did not find any adverse event that led to the discontinuation of Evolocumab. There were no adverse events that could be directly related to Evolocumab. There were no strokes (ischemic or hemorrhagic) and no new-onset Diabetes reported. We do report one case of CV death, caused by chronic heart failure; in this patient, we do not report any coronary event during the time he participated in the study. There was not any death caused by coronary disease during the 96-week follow-up. We did not find a significant occurrence of hospitalization due to unstable angina (1 episode).

The study reported 24 serious adverse events (SAE). We found that people with increased levels of LDL-C (>1mmol/L) had SAE more frequently (p=0.049). However, if we put aside the adverse events related to the secondary endpoint, that is, major CV events, we find that both groups had the same number of SAE (9 for each group). When we used 0.4mmol/L as cutpoint (instead of 1mmol/L), we did not find a statistically significant difference between patients below and above this cutpoint.

When comparing the incidence of neurocognitive dysfunction or an increase of uncontrolled Diabetes Mellitus between patients with very low levels of LDL-C, we didn't find statistically significant differences. We also used 1mmol/L and 0.4mmol/L as cut points. Both had identical results.

Overall safety results are described in Table 4.

Table 4. Overall safety report.

Adverse events reported – no. (%)	n=116
New-Onset Diabetes	0
Hemorrhagic Stroke	0
Neurocognitive Affection	4 (3.4%)
Total secondary endpoint	6 (5.2%)
Hospitalization due to unstable angina	0
Myocardial Infarction	4 (3.4%)
Cardiovascular Death	1 (0.9%)
Caused by a coronary event during the 96-week follow-up	0
Not caused by a coronary event during the 96-week follow-up	1 (0.9%)
Coronary revascularization	0
Ischemic Stroke	0
Serious adverse events	24 (20.7%)

Table 4. Important adverse events occurred during the 96-week follow-up. Results are shown in absolute number and percentage of patients affected.

Discussion

PCSK9 inhibitors have been subject of great interest by the scientific community. A recent meta-analysis presented great results, reporting an LDL-C decrease of approximately 60% across 28 RCTs[1]. These RCTs also exhibited a reduction of other lipid profile measures, just like they exposed a decrease in cardiovascular events[1]. The safety of these new drugs has not been an issue thus far, as major studies have not found statistically significant adverse events, except for injection-site reactions that have been reported as mild, in about 90% of the cases in the FOURIER trial[6]. These results led to their approval from the FDA and EMA for clinical use. Even though recent results have been promising, to have a bigger study sample, follow-up time had to be shorter, leading to the need of continuing to follow these patients to understand the long-term advantages and disadvantages of PCSK9 inhibitors.

One big concern was the possibility of developing neutralizing anti-drug antibodies (nAbs), leading to decreased efficacy, just like what happened with Bococizumab in the SPIRE trial, which led to its premature end[16]. In fact, with Bocolizumab, the SPIRE trial found a 64% decrease in LDL-C levels when they compared week-14 to baseline values. That decrease plummeted to 38.3% in week-104. It was found that patients on Bocolizumab started to develop nAbs.

When we look at our results, we also find significant increases in LDL-C values, namely, between week 12 and week 96 (71% vs 65%) (p=0.001). Nonetheless, when we look at the decreases reported in the FOURIER trial, we realize that our results were even more satisfying, as MS Sabatine reported only 59% decrease in LDL-C[6], while, in our study, after an additional 96-week follow-up, we observed a 65% decrease, 6% more than the FOURIER trial. We must keep in mind that our sample is only a small part of the original FOURIER trial but, with such positive results, we believe there should not be concerns regarding the possibility of nAbs being formed in our sample. It seems that the efficacy of PCSK9 inhibitors in decreasing LDL-C levels continues to be extremely significant after longer periods of time.

One of the big advantages of PCSK9 inhibitors was the possibility of treating Lipoprotein (a) (Lp(a)), a parameter that, not only was proved to be associated with higher coronary risk[2,8,13–15], but is also one that is hard to control, due to the lack of efficient therapeutic options[8,14,15]. In fact, despite studies on various drugs to lower Lp(a) levels, every drug has shown major limitations, those being related to adverse events or lack of clear and consistent results[8]. Moreover, Niacin, which is the one of the most used drugs to control

Lp(a), has been associated with some serious adverse events[8,17] like new-onset diabetes and disturbance in controlling pre-existing diabetes, gastrointestinal adverse events (peptic ulceration and bleeding), myopathy, skin-related adverse events and others[17]. However, PSCK9 have demonstrated great promise, due to the lack of adverse events and significant decreases of Lp(a) levels. Indeed, a recent meta-analysis[14] showed a mean reduction of 21.9% across 27 RCT.

The results of this study may be considered as conflicting results. On one hand, Lp(a) levels did decrease by 18.3% from baseline to week 12 ($p<0.001$). We also found statistical relevant decreases when comparing baseline levels to week 24 and week 72, both presenting decreases of 13.3%. However, after four years of follow-up, we did not find a statistically significant difference in Lp(a) levels.

On the other hand, we found a significant increase in Lp(a) levels when comparing week 12 to each one of the other visits and, furthermore, when comparing baseline levels with week 48 we found no relevant difference ($p=0.103$) as in week 96 ($p=0.053$). These findings raise some questions: can these peaks be explained by something? Is this related only to characteristics of the population studied and, therefore, a lone case? What results are being reported around the world when following other patients during longer periods of time? Can this finding be explained by the fact that we studied only 115 patients?

In fact, there have been reported studies where Lp(a) levels increased slightly with time after an initial decrease, namely FH I and FH II[18] and COMBO II[9], as reported by Daniel Gaudet et colleagues[15]. However, in both studies, when compared to baseline, Lp(a) levels decreases were maintained during the totality of time of follow-up and the slight increases were not reported as statistically significant, a fact that we did not observe in this study. However, one must keep in mind that, in the case of FH I and II, the study focused on patients with familial hypercholesterolemia, therefore, a much different population. However, COMBO II focused on patients without this disease. As far as we know, other RCTs have found significant decreases that were maintained across follow-up time.

Even though we seem to be the only study to report such results, we believe that, in light of what we found, the importance of further studies, with longer follow-up time and different populations, rises, with the objective of understanding how efficient PCSK9 inhibitors really are on lowering Lp(a) levels.

Despite these suboptimal results regarding Lp(a), PCSK9 inhibitors continue to prove efficient in improving lipidic profile, affecting other major measures. In fact, after a median time of four years of follow-up, HDL-C and ApoA1 (the principal component of HDL[19])

showed an increase of 6.1% (p<0.001) and 15.4% (p<0.001), respectively. Triglycerides had a median decrease of 16% (p<0.001). Meanwhile, ApoB, a lipoprotein that has an important role in atherosclerosis[10], had a median decrease of 50% (p<0.001).

Moreover, the efficacy of PCSK9 inhibitors extends beyond raw lipidic measure improvement into practical benefits to its users. MS Sabatine[6] reported a significant difference in the incidence of his primary endpoint (a composite of major coronary events defined as a secondary endpoint in this study) between the Evolocuab group and the control group (9.8% vs 11.3%; p<0.001)[6]. In our study, we found an even lower incidence, only 5.2% of our patients developed a condition that is included in the composite mentioned. Despite this decrease, the difference between the occurrence of the composite in 9.8% of patients in a median of 2.2 years on FOURIER and the 5.2% found in this study follow-up, is not statistically relevant (p=0.064).

When it comes to CV death, the FOURIER trial reported that there was not a statistically significant decrease[6]. At first sight, this result may seem incomprehensible, as we should expect that a decrease in LDL-C levels and the occurrence of coronary events, would lead to better results when comparing CV mortality. But, as we begin to understand how lipidic profile affects clinical outcomes, we start to understand why MS Sabatine did not find any significant decrease in CV death. Really, what happens is that there is a lag between LDL-C reduction and concrete benefit for the patients.

In a 2019 article on the European Heart Journal[7], MS Sabatine details what we should expect when analyzing this relation. First of all, he reports a lag of around six months between LDL-C lowering and a decrease in the incidence of major CV events. That lag leads to a lower benefit during the first year of therapy, when compared to the following years. This fact could help explain why we did not find a statistically significant decrease when comparing the first 2.2 years to the following 1.8 years, as the results of the first year are probably diluted in the next 1.2 years. However, in the FOURIER trial[6], it seems that this issue was not studied, as it is not mentioned any relation between the first year and the next 1.2 years regarding the occurrence of their primary endpoint.

Additionally, he proposes a lag of 1.5 years for the decrease of LDL-C values to make a difference in CV mortality, as it seems to be the point where the mortality curves of control population and statin users population start to separate from each other in the 4S trial[20] and LIPID trial[21]. Therefore, it seems comprehensible that FOURIER, a study with a median follow-up time of 2.2 years, would not report an evident decrease[6]. Likewise, ODYSSE[5] had a median follow-up time of 2.8 years and still found no significant decrease in CV mortality.

In the present study, we report a single death caused by cardiovascular disease, a patient with chronic cardiac insufficiency that died early in the study. There were no coronary events during follow-up time that could have had an important role in the death of this patient. Regardless of these findings, our study does not contain a control population; therefore, we cannot draw any conclusions, even though we only found one death in 116 patients. So, it seems that additional trials, with longer follow-up time, should be performed to prove this association. Fortunately, such trials are already in progress, like HPS4/TIMI65/ORION-4[22] or VESALIUS-CV[23] that expect to follow patients for four to five years, and will certainly help confirm or deny this association and may also clarify how the incidence of coronary events evolves with time. For now, we can only say that current evidence keeps on promising substantial effects.

Whenever we study a new drug, safety is as important (or more important) than its efficacy. This is one of the strongest points of PCSK9 inhibitors.

As mentioned above, one major concern was the possibility of developing nAbs. In the FOURIER trial[6], there were not identified nAbs and only 0.3% developed new binding antibodies. A safety meta-analysis that analyzed, not only FOURIER, but also other trials like OSLER-1, OSLER-2 and DESCARTES, reported no cases of nAbs and rare cases of new binding antibodies[24]. These results are also reported by other authors[2,3,5,25].

In this study, we did not analyze the presence of these molecules. However, as previously mentioned, we found no evidence of its development.

Another concern was the possibility of non-diabetic patients starting to develop diabetes, even though ODYSSEY and FOURIER found no association[5,6]. This concern arises from the fact that there is a correlation between statins and new-onset diabetes[2,24,26–28]. Because of this, more studies have been developed to confirm that PCSK9 inhibitors do not lead to new-onset diabetes. Those studies, along with past ones, have found no such correlation[24,27,28].

In this study, we did not find any case of diabetes in previously healthy patients, supporting the claim that Evolocumab and Alirocumab do not have this unwanted effect.

As studies went on, and some patients started to report LDL-C levels close to 0 mmol/L, a new question started to arise: Could PCSK9 inhibitors cause such a great decrease that it would become a problem for its users? This question becomes more relevant when we take

into consideration that familial hypobetalipoproteinemia, a disease caused by extremely low LDL-C levels, can be caused by mutations leading to PCSK9 inhibition[29]. Fortunately, studies have been performed to answer this question and have shown no increased incidence of symptoms related to hypobetalipoproteinemia in patients on Evolocumab or Alirocumab, namely new-onset diabetes[3,27,28,30], hemorrhagic stroke[30], neurocognitive affection[28,31] and fatty liver[28,30].

In our study, we did not find any case of hemorrhagic stroke or new-onset diabetes, as previously mentioned. We found no evidence of new cases of fatty liver. These results are correlating to the first 2.2 years of following of the FOURIER trial, where none of these events were reported as significant[6].

When it comes to neurocognitive affection, after separating the population into two groups, one where patients reached extremely-low LDL-C levels and the other where patients did not (1mmol/L and 0.4 mmol/l were used as cut points), we found no relation between extremely-low LDL-C levels and neurocognitive affection. The EBBINGHAUS trial, that analyzed this issue on FOURIER's population, reached the same conclusion[31].

We also found that active smokers had less satisfying results in lowering LDL-C, a finding that, as far as we know, hasn't been reported by any author. There may be an explanation to this possible association as, in a 2008 article, Feingold et colleagues[32] established a relation between increased inflammation and an increased expression of PCSK9. Furthermore, across the literature, we can find an exhausting list of articles reporting an association between smoking and inflammation. Despite this, there are not many articles published on the effect of active smoking on PCSK9 inhibitors' efficacy and safety. However, in a 2019 meta-analysis, Raal et all. analyzed 10 ODYSSEY studies (on Alirocumab) and found no significant differences in efficacy between smokers and non-smokers, while using a much bigger sample[33].

These are conflicting results, and we believe that further evidence on this issue should be obtained with further studies.

With such good results in efficacy and lack of safety problems, one might wonder why are these drugs not used more regularly, being only reserved for specific patients, normally, the ones that are not able to control their lipid profile with statins and Ezetimibe, as suggested by the newest guideline on Dyslipidemia treatment[2]. To justify this approach, the authors point to the fact that recent studies, using mid-2018 prices, provide evidence that PCSK9 inhibitors have a low cost-effective relation for the vast majority of patients[34–36]. However,

Evolocumab manufacturers have shown the commitment to reduce the drug's cost[37], which could mean that new patients may be included in the reasonable value, and even in the high value groups, with these new prices. We should expect, for Evolocumab, a smooth decrease until 2020, where prices would be 60% lower than what they were in 2018. Because of this, we may see a more regular use of this drug in the near future.

Limitations

One of the most important limitations to this study is the absence of a control population. This would be helpful to compare lipid profile variations, and how much gain did patients on Evolocumab obtain by using this treatment.

It would also help understand how our patients benefitted from Evolocumab, showing a decrease incidence of our secondary endpoint. However, this wasn't the primary objective of this study.

It would also be helpful in analyzing adverse events.

Regarding adverse events, we did not have access to some other adverse events that are a concern for the scientific community. However, adverse events like tissue vitamin E decreases[24,38], steroid hormone levels[24,38] or liver function worsening[3,24,25,28] have been described as minor and have not been reported in past studies. Nevertheless, it could have been interesting to acquire further evidence of those findings.

Conclusion

In conclusion, we can say that Evolocumab continues to prove to be an effective weapon on dyslipidemia, showing great effectiveness and safety results, which are maintained for long periods of time. Benefit for its patients also correlate to concrete measures, namely the decreased incidence of major CV events.

Despite this, we need to follow these patients long-term to keep confirming the sustainability of these results through time and, on the other hand, to gather further results regarding Lp(a) levels.

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Supplementary Appendix I

Table S1. W96 LDL-C and Lp(a) versus Baseline.

Measure	Median Baseline level [IQR]	Median W96 level [IQR]	Decrease	P-value
LDL-C (mmol/L)	2,23 [1,92; 2,75]	0,83 [0,57; 1,37]	-1.45 (-65 %)	<0.001
Lp (a) (nmol/L)	60,00 [18,00; 183,00]	44,00 [20,00; 161,00]	No statistically relevant decrease found	0.053

Table S1. Median baseline LDL-C (in mmol/L) and Lipoprotein (a) [Lp(a)] in (nmol/L) levels compared to median levels in W96 (4 years of follow-up). Results are shown in median levels and interquartile range; Decreases are shown in median decrease and percentage. We did not find a significant decrease in Lp(a) levels (p=0.053).

Table S2. Baseline Triglycerides, ApoA1 and ApoB versus W96.

Measure (mmol/L)	Median Baseline level [IQR]	Median W96 level [IQR]	Decrease	P-value
Triglycerides	1.25 [0.86-1.85]	1.05 [0.78-1.53]	-0.21 (-17.6 %)	<0.001
ApoA1	1.36 [1.23-1.53]	1.57 [1.39-1.76]	0.2 (14.7 %)	<0.001
ApoB	0.82 [0.72-0.95]	0.42 [0.31-0.56]	-0.41 (-50 %)	<0.001

Table S2. Median baseline triglycerides, ApoA1 (apolipoprotein A) and ApoB (apolipoprotein B) levels compared to median levels in W96 (4 years of follow-up). Results are shown in median levels and interquartile range; variations are shown in median decrease/increase and percentage. Increases are represented by negative results. All values are in mmol/L.

Table S3. Baseline HDL-C versus W96.

Measure (mmol/L)	Mean Baseline level (95% CI)	Mean W96 level (95% CI)	Decrease	P-value
HDL-C	1.28 (+/- 0.39)	1.36 (+/- 0.37)	0.08 (6.25%)	<0.001

Table S3. Mean baseline HDL-C level compared to mean HDL-C levels in W96 (4 years of follow-up). Results are shown in median levels with a 95% confidence interval. The decrease is shown in mean decrease and percentage. All values are in mmol/L.

Table S4. Median ApoA1 levels (mmol/L) across all six timepoints of the study.

ApoA1	Med [Q1; Q3] (mmol/L)	p-value vs Baseline
Rand	1,36 [1,23; 1,53]	
W12	1,43 [1,32; 1,60]	0.067
W24	1,46 [1,28; 1,62]	**0.002**
W48	1,55 [1,39; 1,68]	**<0.001**
W72	1,54 [1,43; 1,70]	**<0.001**
W96	1,57 [1,39; 1,76]	**<0.001**

Table S4. Median Apolipoprotein-A1 levels (mmol/L) across all six timepoints of the study.

P-values show a comparison between said value and Baseline levels. We found other statistical relevant variations:

W12 vs W96; W12 vs W72; W12 vs W48; p<0.001

W24 vs W96; W24 vs W72; W24 vs W48; p<0.001

There were no other statistically relevant oscillations.

Table S5. Median ApoB levels (mmol/L) across all six time points of the study.

ApoB	Med [Q1; Q3] (mmol/L)	p-value vs Baseline
Rand	0,82 [0,72; 0,95]	
W12	0,39 [0,31; 0,50]	**<0.001**
W24	0,37 [0,29; 0,51]	**<0.001**
W48	0,38 [0,29; 0,51]	**<0.001**
W72	0,38 [0,29; 0,56]	**<0.001**
W96	0,42 [0,31; 0,56]	**<0.001**

Table S5. Median Apolipoprotein-B levels (mmol/L) across all six timepoints of the study.

P-values show a comparison between said value and Baseline levels.

There were no other statistically relevant oscillations.

Table S6. Mean HDL-C levels (mmol/L) across all six timepoints of the study.

HDL-C	Mean [95% CI] (mmol/L)	p-value vs Baseline
Rand	1,28 (+/- 0,39)	
W12	1,34 (+/- 0,36)	**<0.001**
W24	1,36 (+/- 0,35)	**<0.001**
W48	1,36 (+/- 0,3)	**<0.001**
W72	1,40 (+/- 0,38)	**<0.001**
W96	1,36 (+/- 0,37)	**<0.001**

Table S6. Median HDL-C levels (mmol/L) across all six timepoints of the study. P-values show a comparison between said value and Baseline levels. We found one other statistical relevant variation: W12 vs W72; p= 0.037 There were no other statistically relevant oscillations.

Table S7. Median Triglyceride levels (mmol/L) across all six timepoints of the study.

TGs	Med [Q1; Q3] (mmol/L)	p-value vs Baseline
Rand	1,25 [0,86; 1,85]	
W12	1,10 [0,82; 1,59]	**0.005**
W24	1,03 [0,78; 1,53]	**<0.001**
W48	0,98 [0,79; 1,41]	**<0.001**
W72	0,98 [0,75; 1,41]	**<0.001**
W96	1,05 [0,78; 1,53]	**<0.001**

Table S7. Median Triglycerides levels (mmol/L) across all six timepoints of the study.
P-values show a comparison between said value and Baseline levels. There were no other statistically relevant oscillations.