The Effects of Glucagon-Like Peptide-1 Receptor Agonists on Atherosclerotic Plaque: Cytokine Profile in Diabetic Individuals

Marcin Hachula[1], Michal Kosowski[1], Marcin Basiak[1], and Boguslaw Okopien[1]

[1]Medical University of Silesia

March 29, 2024

Abstract

Introduction: Type 2 diabetes mellitus (T2DM) represents one of the most pressing global health challenges. The diabetic population has surged dramatically, from 108 million in 1980 to an estimated 529 million in 2021. Hyperglycemia is intricately linked with endothelial dysfunction, which contributes to the development of atherosclerosis, thereby increasing the risk of cardiovascular diseases. Atherosclerotic cardiovascular disease (ASCVD) is closely associated with vulnerable plaques, influenced by numerous cytokines. Consequently, contemporary diabetes treatments must consider pleiotropic effects that mitigate cardiovascular risk. Objectives: This study aimed to investigate the impact of glucagon-like peptide-1 receptor agonists (GLP-1 RAs) on biomarkers indicative of atherosclerotic plaque instability, including pentraxin 3 (PTX3), copeptin (CPC), matrix metalloproteinase-9 (MMP-9), and lipoprotein(a) [Lp(a)]. Patients and Methods: Fifty subjects aged 41–81 years (mean: 60.7) with diagnosed T2DM (median HbA1c: 8.75%), dyslipidemia, and confirmed atherosclerosis via B-mode ultrasound were included. All subjects were eligible to initiate treatment with a GLP-1 RA. Results: Following a 180-day intervention with GLP-1 RAs, our study observed a statistically significant decrease in biochemical markers associated with atherosclerotic plaque instability, including PTX3, CPC, and MMP-9 ($p < 0.001$), as well as Lp(a) ($p < 0.05$). Conclusions: GLP-1 receptor agonists significantly reduce concentrations of PTX3, CPC, MMP-9, and Lp(a), all implicated in plaque vulnerability. This effect may contribute to the reduction of cardiovascular risk among diabetic patients.

The Effects of Glucagon-Like Peptide-1 Receptor Agonists on Atherosclerotic Plaque: Cytokine Profile in Diabetic Individuals

Marcin Hachuła[1], Michał Kosowski[1], Marcin Basiak*[1], Bogusław Okopień[1]

1.Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Medyków 18, 40-752 Katowice, Poland

Correspondence to: Marcin Basiak M.D., Ph.D., Assoc. Prof.: Department of Internal Medicine and Clinical Pharmacology, Medical University of Silesia, Medyków 18, 40-752 Katowice, Poland, phone: +48-32-208-85-10,*mbasiak@sum.edu.pl*.

Conflicts of Interest: The authors declare no conflict of interest.

Abstract:

Introduction : Type 2 diabetes mellitus (T2DM) represents one of the most pressing global health challenges. The diabetic population has surged dramatically, from 108 million in 1980 to an estimated 529 million in 2021. Hyperglycemia is intricately linked with endothelial dysfunction, which contributes to the development of atherosclerosis, thereby increasing the risk of cardiovascular diseases. Atherosclerotic cardiovascular disease (ASCVD) is closely associated with vulnerable plaques, influenced by numerous cytokines. Consequently, contemporary diabetes treatments must consider pleiotropic effects that mitigate cardiovascular risk.

Objectives: This study aimed to investigate the impact of glucagon-like peptide-1 receptor agonists (GLP-1 RAs) on biomarkers indicative of atherosclerotic plaque instability, including pentraxin 3 (PTX3), copeptin (CPC), matrix metalloproteinase-9 (MMP-9), and lipoprotein(a) [Lp(a)].

Patients and Methods: Fifty subjects aged 41–81 years (mean: 60.7) with diagnosed T2DM (median HbA1c: 8.75%), dyslipidemia, and confirmed atherosclerosis via B-mode ultrasound were included. All subjects were eligible to initiate treatment with a GLP-1 RA.

Results: Following a 180-day intervention with GLP-1 RAs, our study observed a statistically significant decrease in biochemical markers associated with atherosclerotic plaque instability, including PTX3, CPC, and MMP-9 ($p < 0.001$), as well as Lp(a) ($p < 0.05$).

Conclusions: GLP-1 receptor agonists significantly reduce concentrations of PTX3, CPC, MMP-9, and Lp(a), all implicated in plaque vulnerability. This effect may contribute to the reduction of cardiovascular risk among diabetic patients.

Keywords: GLP-1 receptor agonist; Diabetes mellitus; Pentraxin 3; MMP-9, Copeptin, Lipoprotein (a); semaglutide; dulaglutide

INTRODUCTION

The global diabetic population is on the rise, estimated to have increased from 108 million in 1980 to 529 million in 2021 [1]. Individuals with diabetes face a heightened risk of complications, especially macrovascular complications including coronary artery disease, cerebrovascular disease, and peripheral artery disease, which constitute major causes of mortality, accounting for over 50% of deaths in diabetic patients [2]. The development of atherosclerosis is responsible for the onset of cardiovascular diseases [3]. Hyperglycemia is strongly linked to endothelial dysfunction, which not only initiates the formation of atherosclerotic plaques but also contributes to their progression and instability [4]. The pathomechanism of atherosclerosis is highly complex, involving multiple stages such as initiation due to endothelial dysfunction, inflammatory cell migration, atherosclerotic plaque formation, and eventual plaque rupture. Numerous cytokines play key roles in each of these stages [5]. The clinical implications of atherosclerotic cardiovascular disease (ASCVD) is associated with vulnerable plaques. This term encompasses different phenotypes of unstable atherosclerotic plaques, such as plaque rupture, intraplaque hemorrhage, erosion and plaque fissuring [6]. Cytokines involved in the vulnerability of atherosclerotic plaques include e.g. pentraxin 3 (PTX3), lipoprotein(a) [Lp(a)], copeptin (CPC), and matrix metalloproteinase 9 (MMP9).

Pentraxin 3 is emerging as a promising immunoinflammatory marker for evaluating cardiovascular risk. Within the pentraxin family, which comprises acute-phase proteins characterized by a pentameric structure, C-reactive protein (CRP) is one of the members. Importantly, while CRP is predominantly synthesized in the liver, PTX3 is locally produced and released by various cell types, particularly monocytes/macrophages. The production of PTX3 is stimulated by pro-inflammatory cytokines such as Interleukin 1 (IL-1), tumor necrosis factor α (TNFα), and oxLDL [7]. The distinctive capability of pentraxin 3 to regulate local inflammation involving macrophages and smooth muscle cells has prompted research into its role in the pathogenesis of atherosclerosis and cardiovascular disease. The observed positive correlation between PTX3 concentration and the risk of adverse outcomes in patients with coronary artery disease (CAD) suggests that PTX3 could be a valuable biomarker for cardiovascular disease [8, 9]. Furthermore, the plasma PTX3 concentration is correlated with plaque vulnerability evaluated by optical coherence tomography in individuals with coronary artery disease [10, 11].

The precursor peptide preprovasopressin produced in the hypothalamus is responsible for the release of arginine vasopressin (AVP) and an equal amount of copeptin. Their secretion occurs, among other factors, in response to stress and affects the regulation of the endocrine response of the hypothalamo-pituitary-adrenal (HPA) axis. The precise function of copeptin remains elusive. In contrast to AVP, measuring plasma copeptin concentration is more accessible. Therefore, its primary role lies in serving as an indirect indicator of the circulating plasma levels of AVP [12]. Copeptin could be a promising new marker for diagnosing acute

cardiovascular events. Numerous studies indicate that, when evaluated alongside cardiac troponin (cTn), copeptin is effective in swiftly ruling out myocardial infarction. Furthermore, in cases of stroke, myocardial infarction, or heart failure, it can be employed for risk stratification and prognosis assessment [13].

Lp(a) is an independent risk factor for ASCVD [14]. Lipoprotein(a) plays a role at various stages of atherosclerosis. Within the endothelium, it undergoes more pronounced oxidation than LDL, thus intensifying the action of adhesion molecules. Lp(a) increases the synthesis of other pro-inflammatory cytokines such as IL-1, IL-6, and TNFα. Lipoprotein(a) is also involved in the instability of atherosclerotic plaques. Its prothrombotic and antifibrinolytic effects contribute to intravascular thrombotic processes [15, 16, 17]. In recent years, a correlation has been established between elevated plasma lipoprotein(a) levels and the presence of vulnerable atherosclerotic plaques in patients experiencing acute cardiovascular events [18, 19].

Matrix metalloproteinases (MMPs) are enzymes crucial for remodeling the extracellular matrix and facilitating leukocyte recruitment to inflammatory sites, thereby serving as key regulators of the inflammatory process. Consequently, excessive or imbalanced secretion of MMP-9 is linked to tissue damage in inflammation [20]. MMP-9 contributes to the progression of arteriosclerosis. Numerous studies indicate a correlation between elevated plasma levels of matrix metalloproteinases-9 and an increased risk of plaque rupture and acute cardiovascular events [21].

Hence, inhibiting the progression of atherosclerosis and preventing its instability has huge clinical significance in enhancing the prognosis for individuals with diabetes. In the treatment of type 2 diabetes, there is an increased use of 'new hypoglycemic drugs,' such as glucagon-like peptide 1 receptor agonists (GLP-1 RA). These medications, besides affecting blood glucose levels through pleiotropic effects, also influence various cardiac risk factors [22]. In recent years, several randomized clinical trials of GLP-1 RA have demonstrated a substantial reduction in cardiovascular risk [23, 24, 25, 26]. The mechanism behind this phenomenon remains incomprehensible. In connection with the decrease in cardiovascular events among patients diagnosed with ASCVD, there was a hypothesis that these drugs affect atherogenesis [27].

Consequently, the objective of our study was to explore the impact of GLP-1 RA on biomarkers of atherosclerotic plaque instability, including PTX3, CPC, MMP-9, and Lp(a).

2. Materials and Methods

2.1. Study Population

Out of 91 patients, we included 50 individuals aged 41–81 (mean: 60.7) in the study. All subjects were diagnosed with type 2 diabetes mellitus, dyslipidemia, and confirmed atherosclerosis based on B-mode ultrasound common carotid intima-media thickness. The medical experiment took place from January 2022 to September 2023. Individuals meeting highly specific and narrow inclusion and exclusion criteria were deemed eligible to participate in the study. Each patient provided informed consent in accordance with the Declaration of Helsinki. All information about the subjects was anonymized. Patients were recruited at the Department of Internal Medicine and Clinical Pharmacology in Katowice, Poland, and through referrals from the Mysłowice and Imielin diabetes outpatient departments. The study protocol was approved by the Bioethical Committee of the Medical University of Silesia (PCN/CBN/0052/KB1/45/I/22). Type two diabetes is one of the world's biggest health problems., either semaglutide (n = 16) or dulaglutide (n = 34), at a typical hypoglycemic dose- patients received the maximum tolerated doses, unless glycemic control was achieved with lower doses. The drug was administered every week at the same time of day. The choice of treatment was determined by drug availability on the Polish market. During the intervention, the GLP-1 receptor agonist therapy, as well as other medications, was not modified. The intervention lasted for a duration of 180 days. Figure 1 shows the flowchart of the study.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria were: type 2 diabetes; dyslipidemia, defined as plasma total cholesterol (TC) > 200 mg/dL and/or triglycerides (TG) > 150 mg/dL; the presence of atherosclerotic plaque in the common carotid artery, confirmed by ultrasound examination.

Patients were excluded from the study in the following cases: type 1 diabetes, cardiac disorders such as exacerbation of chronic heart failure and unstable coronary artery disease, a history of percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), or stroke within 3 months before starting the study. Furthermore, exclusion criteria included chronic pancreatitis, acute exacerbation of autoimmune disorders, pregnancy, breastfeeding period, uncompensated thyroid disease, alcoholism, any acute and chronic inflammatory processes, including COVID-19 infection within 4 weeks before study and also any acute infection defined as an increase in CRP values $>$ 5 mg/dL or leukocytosis, chronic kidney disease below stage G3b with glomerular filtration rate (eGFR) $<$ 45 mL/min/1.73 m2, acute and chronic liver diseases expressed as an increase in transaminases above 3 times the norm, or diagnosed chronic viral hepatitis in medical history. After the intervention, all subjects were interviewed once again. They were also excluded if, within the last 6 months, they increased their physical activity, changed their type of diet, modified their treatment, or started therapy with a new drug proven to affect lipid serum levels or with a known pleiotropic effect (e.g., statins, fibrates, ezetimibe, niacin, non-selective beta-blockers, metformin, sodium-glucosium like transporter 2 (SGLT2) inhibitors, or ursodeoxycholic acid). Additionally, exclusion criteria applied if they experienced a coronary or stroke incident or suffered a severe infection.

2.3. Laboratory and Anthropometric Measurements

All measurements were taken before study enrollment and after 6 months of treatment by a physician. Body weight and height were measured following standard procedures, and body mass index (BMI) was calculated in kg/m2. Waist and hip circumferences were measured at the typical locations, and the waist/hip ratio (WHR) was computed. Arterial blood pressure (BP) was measured twice in the sitting position in the arm without vascular access using the Omron M400 Intelli IT automatic device. To estimate the eGFR, the CKD-EPI formula was applied, and the values were presented in mL/min/1.73 m2. Routine laboratory measurements were performed in the certified laboratory, and venous blood samples were collected after an overnight 12-hour fasting period at 8 a.m., both before the treatment and after 180 days of intervention. The plasma levels of cytokines were assessed using commercially available Enzyme-linked immunosorbent assay (ELISA) kits, as described by the manufacturers: Lp(a) (*10-1106-01* , Mercodia AB, Sweden). MMP-9 and CPC: (SEA553Hu, CEA365HU; Cloud-Clone Corporation, Houston, Texas, USA). PTX3 (RD191477200R, BioVendor, Brno, Czech Republic).

2.4. Arteriosclerotic Plaque Examination

The examination of the carotid arteries and the assessment of complex intima media thickness (C-IMT) in the extracranial segment was performed using B-mode ultrasound with a linear probe at a frequency of 7.5–10 MHz on a Hitachi Aloka F37 ultrasound machine. According to the Atherosclerosis Risk in Communities Study, the C-IMT was evaluated 3 times, and the average score was taken into account. The measurement was performed in the distal common carotid (1 cm proximal to the carotid bulb). For confirmation of atherosclerotic plaque in the carotid artery, we assumed a thickness of the C-IMT complex $>$ 1.5 mm or the presence of plaque, in accordance with the guidelines.

2.5. Statistical analysis

The data was processed using Statistica TIBCO Software Inc. (2017) version 13.3 soft-ware, which was licensed by the Medical University of Silesia in Katowice. To assess the normality of distributions, we used the Shapiro-Wilk test. Values are presented as means and 95% confidence intervals or medians with Q1–Q3 values. To compare quantitative variables the t-test for independent means and t-test for dependent means were used. To compare independent variables with an abnormal distribution U Mann- Whitney test was used. For dependent variables we used Wilcoxon test. We assumed a p-value of less than 0.05 was statistically significant. We also use Spearman rank correlation to assess the relationship between variables. We assumed a p-value of less than 0.05 was statistically significant.

3. RESULTS

3.1. Study and Control Group Characteristics

The study group consisted of 50 patients with a mean age of 60.8 ± 9.5 years, including 27 (54%) women. Overall, 24% were overweight (BMI > 25 kg/m2) and 70% were obese (BMI > 30 kg/m2). Average WHR was: 0.975. All subjects were diagnosed with type 2 diabetes mellitus (median glycated haemoglobin (HbA1c): 8.75%; average duration time is 10.5 years) and dyslipidemia. The concomitant diseases included hypertension (80%), chronic kidney disease (18%—all in stage G3a), hypothyroidism (14% everyone with normalized (thyroid-stimulating hormone (TSH) value) and heart failure with reduced ejection fraction (8%). For diabetes treatment, patients chronically received metformin (98%), sulfonylurea (44%), SGLT2 inhibitors (20%), dipeptidyl peptidase 4 (DPP-4) inhibitors (8%) and insulin (28%). Full treatment data are available in the Table 1. The medications used by the patient were not changed during the intervention. At baseline, serum median levels of total cholesterol was 166.5 mg/mL, low-density lipoprotein-cholesterol (LDL-C) was 83 mg/dL, high-density lipoprotein-cholesterol (HDL-C) was: 49.5 mg/dL and triglycerides (TG) was 167.9 mg/dL. Mean levels of alanine transaminase (ALT) and aspartate transaminase (AST) were 34.4 U/L and 32.5 U/L respectively, and the median systolic blood pressure (SBP) was 135 mmHg. There were 18% active smokers and none of the patients abused alcohol. In total, 20 subjects (40%) met the World Health Organization (WHO) criteria for physical activity.

The control group consisted of 26 sex-matched patients with a mean age of 33.08 ± 5.45 years, including 13 (50%) women. Overall, 19% were overweight (BMI > 25 kg/m2) and no one was obese (BMI > 30 kg/m2). Average WHR was: 0.83. All subjects were not diagnosed with any concomitant diseases. Baseline characteristics and comparison of metabolic parameters between study and control group are presented in Tables 2 and 3.

3.2 Comparison of the levels of cytokines responsible for atherosclerotic plaque instability between the study and healthy groups

We observed statistically significant lower concentrations of pentraxin 3, copeptin, and matrix metalloproteinase 9 (MMP-9) in the healthy control group compared to the study group before treatment ($p<0.001$). No statistical significance was observed for lipoprotein(a). Detailed results are presented in Table 3 and Figures 2-5.

3.3 Biochemical effect after 180 days treatment.

In the study group after treatment, we obtained statistically significant reduction in anthropometric parameters including BMI ($p <0.001$), on average, patients lost 5.1 kg of weight. Substantial differences in decreasing blood pressure for SBP ($p < 0.001$) and DPB ($p < 0.001$) were noted. Lower concentration of fasting glucose and HbA1c average reduction of 1.12% (mean: 7.56%) ($p <0.001$) was observed. 17 (34%) individuals achieved the target value for HbA1c. Regarding the lipid profile, patients also experienced a decrease in LDL fraction, TG, and non-HDL cholesterol, along with an elevation in HDL fraction; nevertheless, these alterations did not reach statistical significance. Furthermore, we obtained a statistically significant decrease in fibrosis-4 score (FIB-4) ($p <0.001$) and De-Ritis Ratio ($p <0.05$) Detailed results are presented in Table 4.

3.4 Effect of 180 days treatment on biochemical markers of atherosclerotic plaque vulnerability

After a 180-day intervention with GLP-1 RA in our study group, we observed a statistically significant decrease in biochemical markers associated with atherosclerotic plaque instability, including PTX3, CPC, MMP-9 ($p < 0.001$), and ($p < 0.05$) for Lp(a). The results are presented in Figures 6-9

3.5 Correlation between changes in biochemical parameters and cytokine concentrations.

An important observation is that changes in the concentrations of the studied cytokines do not correlate with changes in HbA1C value and body weight, except for lipoprotein(a). Details of the correlations we investigated are shown in Table 5.

4. DISCUSION

The presented study demonstrates the beneficial effects of GLP-1 receptor agonists on various factors increasing cardiovascular risk in patients with type 2 diabetes (T2D). We assessed anthropometric parameters, biochemical parameters, and serum concentrations of cytokines influencing plaque vulnerability in a controlled group and in individuals treated with GLP-1 receptor agonists before and after treatment.

As type 2 diabetes advances over the years, arteries become more susceptible to the development of atherosclerosis and the onset of cardiovascular complications, such as myocardial infarction [28]. In our study, individuals with dyslipidemia and type 2 diabetes mellitus exhibited a statistically significant elevation in levels of cytokines associated with atherosclerotic plaque instability, including PTX3, CPC, and MMP-9, among others, when compared to a healthy group. This finding aligns with similar results observed in numerous other studies [29, 30, 31]. In our study, we demonstrated that there was no statistical difference in the baseline level of lipoprotein(a) in the study group with diabetes compared to the group of healthy subjects. Furthermore, the mean concentration level in the group of healthy subjects was higher. Conflicting reports exist in the literature regarding lipoprotein(a) levels in individuals with diabetes, and their consensus reveals a certain paradox, as these concentrations are generally reported to be lower in people with diabetes than healthy people [32, 33]. Insulin may be responsible for this phenomenon, and it has been shown in both clinical and experimental model studies that insulin directly reduces Lp(a) levels through a negative response to the LPA gene [34].

Large randomized clinical trials (RCT) of GLP-1 RA, including PIONEER, REWIND, SUSTAIN and LEADER, have shown their positive effects on reducing cardiovascular risk [23, 24, 25, 26]. The mechanism behind this action remains unexplained and is the focus of numerous ongoing studies worldwide. Our hypothesis is that GLP-1 receptor agonists influence various stages of atherogenesis. In another of our recently published papers, we illustrated that after 180 days of therapy, GLP-1 RA lowers the concentration of proinflammatory cytokines responsible for initiating atherosclerotic plaque [35]. In this study, our objective was to examine the impact of treatment with a GLP-1 receptor agonist on the process of atherosclerotic plaque stabilization by assessing plasma markers indicative of plaque vulnerability.

In recent years, pentraxin 3, actively participating in various stages of atherosclerosis development, has gained attention among researchers as a new biochemical marker for cardiovascular risk [9]. Numerous studies have shown that increased PTX3 levels have been associated with the presence of high-risk plaque [10, 11, 36] and acute cardiovascular events [8, 37, 38, 39, 40]. In our study, therapeutic intervention with semaglutide and dulaglutide resulted in a decrease in PTX3 serum levels in patients diagnosed with diabetes and dyslipidemia. In the available literature, there are reports of a positive effect of liraglutide on reducing the expression of PTX3 in primary human epithelial cells taken from the umbilical vein [41]. In animal model studies, administration of exenatide reduced plasma PTX3 levels in rats [42]. In the same human study conducted by Suzuki et al. noted a rise in PTX3 levels among patients following liraglutide treatment [43]. Discrepancies in the findings could be attributed to the inclusion of participants with parallel dyslipidemia and ultrasound-confirmed atherosclerosis in our study group. Notably, the study by Suzuki et al. lacked information about cardiovascular disease in their participant cohort. Pentraxin 3 and its heightened levels are linked with atherosclerotic cardiovascular disease [9].

The role of MMP-9 in atherosclerotic plaque instability is well understood and widely studied. There are numerous reports showing that plasma MMP-9 levels may be a prognostic factor for future adverse cardiovascular events [44]. Our 180-day treatment intervention involving GLP-1 receptor agonists led to a statistically significant reduction in metalloproteinase 9 plasma levels in patients with diabetes and dyslipidemia. The results of the studies conducted so far on animal models align with our findings [45, 46, 47, 48]. To the best of our knowledge, there are no comparative studies demonstrating the impact of GLP-1 receptor agonists on MMP-9 levels in patients with high cardiovascular risk.

Anything that disrupts the body's homeostatic balance, such as an acute cardiovascular incident, activates the HPA axis, leading to an increase in concentrations of the stress hormone. One of the major hypothalamic stress hormones, stimulated by various stressors, is vasopressin (AVP), along with copeptin, which is released in an equimolar ratio [49]. As a biomarker, copeptin has been well studied in cardiovascular disease obtaining

very satisfying results [13, 50]. In our study, we observed a statistically significant reduction in copeptin levels among patients who were treated with GLP-1 RA. In their work, Svenja Leibnitz et.al. also obtained a statistical decrease in copeptin levels in healthy volunteers and in patients with polydipsia treated with dulaglutide [51]. On the other hand, in this work, also in healthy volunteers, intervention with another analog - exenatide - was not associated with a significant decrease in copeptin levels - it is worth noting that the blood drawn for the study came from 6 people [52]. Treatment with liraglutide had no statistically significant effect in women with polycystic ovary syndrome (PCOS) on copeptin levels [53]. It is essential to consider that GLP-1 RA may reduce water intake, potentially influencing copeptin concentrations in the organism [54]. The findings from our study contribute to the investigation of copeptin's potential role as a biomarker for cardiovascular risk. Based on our current understanding, there are no comparative studies clarifying the influence of GLP-1 RA on copeptin levels in diabetic patients.

In patients with coronary artery disease, a link between increased lipoprotein(a) levels and atherosclerotic plaque instability has been proven [18, 19, 55, 56]. Several large RCTs, including JUPITER, LIPID and AIM-HIGH, have found that Lp(a) remains a predictor of CVD events, even in patients treated with potent statins who achieve low LDL concentrations [34, 57]. Therefore, it is important in modern therapy to use drugs with a pleiotropic mechanism, which will further reduce cardiovascular risk by decreasing lipoprotein(a) levels. In our study group, therapy with GLP-1 RA resulted in a significant decrease in lipoprotein(a). It is worth noting that we did not obtain a significant decrease in terms of lipidogram values. Similar results to ours were obtained by Bechlioulis A. et al. Also, in patients with type 2 diabetes treated with liraglutide, he obtained an improvement in Lp(a) values [58]. Similar observations in patients with diabetes were made by Steven R et al. The difference was the drug used - exenatide [59]. On the other hand, Ariel D et al. in patients with an overdiabetic state, did not observe a change in lipoprotein(a) concentration during liraglutide therapy [60]. There is a significant need for large-scale cross-sectional studies to definitively assess changes in lipoprotein(a) levels during treatment with GLP-1 RA, given the high baseline cardiovascular risk in patients with diabetes. And the multi-pronged benefit of using these drug groups.

Most importantly, in our study, we simultaneously performed a Sperman correlation, showing that the decrease in the concentration of the above cytokines was independent of weight loss and changes in the amount of hemoglobin glycated. Supporting the hypothesis that GLP-1 receptor agonists decrease cardiovascular risk through a mechanism unrelated to the positive anthropometric and metabolic alterations [61].

Our study is subject to various limitations. Initially, the study included a relatively small sample size of only 50 patients. Additionally, there was a lack of a control group receiving either a placebo or an active comparator. Furthermore, during the planning phase, our intention was to exclusively utilize semaglutide; however, challenges with supply and availability in the Polish market compelled us to switch to dulaglutide during the course of the study. Finally, it is important to note that this study exclusively focused on patients from the Upper Silesia region of Poland, and the outcomes may vary based on factors such as residence, race, and environmental conditions.

5. Conclusion

To sum up, in addition to the already known positive effect of GLP-1RA on the metabolic and anthropometric parameters of diabetic patients treated with them, these drugs significantly reduce the concentrations of PTX3, CPC, MMP-9, and Lp(a) which are involved in plaque vulnerability. This effect could be responsible for reducing cardiovascular risk in patients with diabetes. Additional studies, especially randomized clinical trials are needed to indisputably assess the impact of these drugs on the development of cardiovascular diseases and the risk of cardiovascular events.

Author Contributions

Conceptualization, M.H. and M.B.; methodology, M.H.; resources, M.H.; statistical analysis, M.K.; writing—original draft preparation, M.H. writing—review and editing, M.K. and M.B., supervision, B.O.; project administration, M.H. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Medical University of Silesia, grant number PCN-1-227/N/2/O

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Bioethical Committee of the Medical University of Silesia PCN/CBN/0052/KB1/45/I/22.

Acknowledgments

The authors gratefully acknowledge Grzegorz Hachuła for helping with the recruiting process, Jarosława Sprada, Kinga Banasik and Danuta Chwiedacz for laboratory maintenance, and Sabina Ryl for language support.

References:

1. GBD 2021 Diabetes Collaborators. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 202; 402: 203-234.
2. Wong ND, Sattar N. Cardiovascular risk in diabetes mellitus: epidemiology, assessment and prevention. Nat Rev Cardiol. 2023; 20: 685-695.
3. Frostegård J. Immunity, atherosclerosis and cardiovascular disease. BMC Med. 2013;11:117.
4. Maruhashi T, Higashi Y. Pathophysiological Association between Diabetes Mellitus and Endothelial Dysfunction. Antioxidants (Basel). 2021; 10: 1306.
5. Jebari-Benslaiman S, Galicia-García U, Larrea-Sebal A, et.al. Pathophysiology of Atherosclerosis. Int J Mol Sci. 2022; 23: 3346
6. Lutgens E, van Suylen RJ, Faber BC, et.al Atherosclerotic plaque rupture: local or systemic process? Arterioscler Thromb Vasc Biol. 2003; 23: 2123-30
7. Mantovani A, Garlanda C, Bottazzi B, et. al. The long pentraxin PTX3 in vascular pathology. Vascul Pharmacol. 2006; 45: 326-30
8. Kamarullah W, Nurcahyani, Multazam RB, Josephine CM. Pentraxin 3 concentration is associated with poor outcomes in patients with coronary artery disease: a systematic review and dose-response meta-analysis. Acta Cardiol. 2022; 77: 385-394.
9. Ye X, Wang Z, Lei W, et.al. Pentraxin 3: A promising therapeutic target for cardiovascular diseases. Ageing Res Rev. 2023; 93:102163.
10. Tazaki R, Tanigawa J, Fujisaka T, et.al. Plasma Pentraxin3 Level Is Associated With Plaque Vulnerability Assessed by Optical Coherence Tomography in Patients With Coronary Artery Disease. Int Heart J. 2016; 57:18-24.
11. Koga S, Ikeda S, Yoshida T, et.al. Elevated levels of systemic pentraxin 3 are associated with thin-cap fibroatheroma in coronary culprit lesions: assessment by optical coherence tomography and intravascular ultrasound. JACC Cardiovasc Interv. 2013; 6: 945-54.
12. Christ-Crain M. Vasopressin and Copeptin in health and disease. Rev Endocr Metab Disord. 2019; 20: 283-294.
13. Mu D, Cheng J, Qiu L, Cheng X. Copeptin as a Diagnostic and Prognostic Biomarker in Cardiovascular Diseases. Front Cardiovasc Med. 2022; 9: 901990.
14. Alhomoud IS, Talasaz A, Mehta A, et.al. Role of lipoprotein(a) in atherosclerotic cardiovascular disease: A review of current and emerging therapies. Pharmacotherapy. 2023; 43:1051-1063
15. Rehberger Likozar A, Zavrtanik M, Šebeštjen M. Lipoprotein(a) in atherosclerosis: from pathophysiology to clinical relevance and treatment options. Ann Med. 2020; 52: 162-177.
16. Kronenberg F, Mora S, Stroes ESG, et.al. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. Eur Heart J. 2022; 43 :3925-3946
17. Lampsas S, Xenou M, Oikonomou E, et.al. Lipoprotein(a) in Atherosclerotic Diseases: From Pathophysiology to Diagnosis and Treatment. Molecules. 2023; 28: 969.

18. Kato A, Kinoshita D, Nagata T, et.al. Lipoprotein (a) levels and vulnerable characteristics in nonculprit plaque in patients with acute coronary syndrome. Int J Cardiol Heart Vasc. 2022; 43: 101120.
19. Muramatsu Y, Minami Y, Kato A, et.al. Lipoprotein (a) level is associated with plaque vulnerability in patients with coronary artery disease: An optical coherence tomography study. Int J Cardiol Heart Vasc. 2019; 24: 100382
20. Jönsson, S.; Lundberg, A.K.; Jonasson, L. Overexpression of MMP-9 and its inhibitors in blood mononuclear cells after myocardial infarction–is it associated with depressive symptomatology? PLoS One. 2014; 25: e105572.
21. Gough PJ, Gomez IG, Wille PT, Raines EW. Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice. J Clin Invest 2006; 116: 59-69.
22. Masson W, Lavalle-Cobo A, Lobo M, et.al. Novel antidiabetic drugs and risk of cardiovascular events in patients without baseline metformin use: a meta-analysis. Eur J Prev Cardiol. 2021; 28: 69-75.
23. Marso SP, Daniels GH, Brown-Frandsen K, et.al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med. 2016; 375: 311-22.
24. Husain M, Birkenfeld AL, Donsmark M et.al. Oral Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med. 2019; 381: 841-851.
25. Marso SP, Bain SC, Consoli A, et.al Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med. 2016; 375: 1834-1844.
26. Gerstein HC, Colhoun HM, Dagenais GR, et.al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. Lancet. 2019; 394: 121-130.
27. Marx N, Husain M, Lehrke M, et.al. GLP-1 Receptor Agonists for the Reduction of Atherosclerotic Cardiovascular Risk in Patients With Type 2 Diabetes. Circulation. 2022; 146: 1882-1894.
28. Steen Carlsson K, Faurby M, Nilsson K, Wolden ML. Atherosclerotic Cardiovascular Disease in Type 2 Diabetes: A Retrospective, Observational Study of Economic and Clinical Burden in Sweden. Diabetes Ther. 2023; 14: 1357-1372.
29. Jenny NS, Blumenthal RS, Kronmal RA, et.al. Associations of pentraxin 3 with cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. J Thromb Haemost. 2014; 12: 999-1005.
30. Szmygin H, Szydełko J, Matyjaszek-Matuszek B. Copeptin as a novel biomarker of cardiometabolic syndrome. Endokrynol Pol. 2021;72: 566-571
31. Stepień E, Dworzański J, Dworzańska A, et.al. Serum Level of MMP-3 and MMP-9 in Patients with Diabetes Mellitus Type 2 Infected with Epstein-Barr Virus. Int J Mol Sci. 2022; 23: 13599.
32. Kostner KM, Kostner GM. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus. Int J Mol Sci. 2022; 23: 3584.
33. Ward NC, Vickneswaran S, Watts GF. Lipoprotein (a) and diabetes mellitus: causes and consequences. Curr Opin Endocrinol Diabetes Obes. 2021; 28: 181-187.
34. Tsimikas S. Lipoprotein(a): novel target and emergence of novel therapies to lower cardiovascular disease risk. Curr Opin Endocrinol Diabetes Obes. 2016; 23:157-64.
35. Hachuła M, Kosowski M, Ryl S, et.al . Impact of Glucagon-Like Peptide 1 Receptor Agonists on Biochemical Markers of the Initiation of Atherosclerotic Process. Int J Mol Sci. 2024; 25:1854.
36. Conte E, Andreini D, Magnoni M et.al. Association of high-risk coronary atherosclerosis at CCTA with clinical and circulating biomarkers: Insight from CAPIRE study. J Cardiovasc Comput Tomogr. 2021; 15: 73-80.
37. Zagidullin N, Motloch LJ, Gareeva D, et.al. Combining Novel Biomarkers for Risk Stratification of Two-Year Cardiovascular Mortality in Patients with ST-Elevation Myocardial Infarction. J Clin Med. 2020; 9: 550.
38. Wang Y, Zhao X, Zhou P, et.al. Plasma Pentraxin-3 Combined with Plaque Characteristics Predict Cardiovascular Risk in ST-Segment Elevated Myocardial Infarction: An Optical Coherence Tomography Study. J Inflamm Res. 2021; 14: 4409-4419.
39. Dejanović VV, Stevuljević JK, Vukašinović A, et.al. Oxidative Stress and Inflammatory Markers PTX3, CypA, and HB-EGF: How Are They Linked in Patients With STEMI? Angiology. 2020; 71: 713-720.

40. Yi L, Tang J, Shi C, et.al. Pentraxin 3, TNF-α, and LDL-C Are Associated With Carotid Artery Stenosis in Patients With Ischemic Stroke. Front Neurol. 2020; 10: 1365.
41. Shiraki A, Oyama J, Komoda H, et.al. The glucagon-like peptide 1 analog liraglutide reduces TNF-α-induced oxidative stress and inflammation in endothelial cells. Atherosclerosis. 2012; 221: 375-82.
42. Artunc-Ulkumen B, Pala HG, Pala EE, et.al. Exenatide improves ovarian and endometrial injury and preserves ovarian reserve in streptozocin induced diabetic rats. Gynecol Endocrinol. 2015; 1: 196-201.
43. Suzuki D, Toyoda M, Kimura M, et.al. Effects of liraglutide, a human glucagon-like peptide-1 analogue, on body weight, body fat area and body fat-related markers in patients with type 2 diabetes mellitus. Intern Med. 2013; 52: 1029-34.
44. Li T, Li X, Feng Y, et.al. The Role of Matrix Metalloproteinase-9 in Atherosclerotic Plaque Instability. Mediators Inflamm. 2020; 2020: 3872367.
45. Yang G, Lei Y, Inoue A, et.al. Exenatide mitigated diet-induced vascular aging and atherosclerotic plaque growth in ApoE-deficient mice under chronic stress. Atherosclerosis. 2017; 264: 1-10.
46. Wolak M, Staszewska T, Juszczak M, et.al. Anti-inflammatory and pro-healing impacts of exendin-4 treatment in Zucker diabetic rats: Effects on skin wound fibroblasts. Eur J Pharmacol. 2019; 842: 262-269
47. Cardoso LEM, Marinho TS, Martins FF, et.al. Treatment with semaglutide, a GLP-1 receptor agonist, improves extracellular matrix remodeling in the pancreatic islet of diet-induced obese mice. Life Sci. 2023; 319: 121502.
48. Ding HX, Dong NX, Zhou CX, et.al. Liraglutide Attenuates Restenosis After Vascular Injury in Rabbits With Diabetes Via the TGF-β/Smad3 Signaling Pathway. Altern Ther Health Med. 2022; 28: 22-28.
49. Katan M, Christ-Crain M. The stress hormone copeptin: a new prognostic biomarker in acute illness. Swiss Med Wkly. 2010; 140: w13101.
50. Schill F, Persson M, Engström G, et.al. Copeptin as a marker of atherosclerosis and arteriosclerosis. Atherosclerosis. 2021; 338: 64-68.
51. Leibnitz S , Christ-Crain M, Winzeler B. Effects of glucagon-like peptide-1 receptor agonists on copeptin in healthy volunteers and patients with primary polydipsia. Presented at ESE Young Endocrinologists and Scientists (EYES) 2023. Endocrine Abstracts (2023) 93 OC10
52. Heinla K, Vasar E, Reppo I, et.al. GLP-1 Receptor Agonists Induce Growth Hormone Secretion in Healthy Volunteers. Diabetes Ther. 2023; 14: 777-786.
53. Frøssing S, Nylander M, Kistorp C, et.al. Effect of liraglutide on atrial natriuretic peptide, adrenomedullin, and copeptin in PCOS. Endocr Connect. 2018; 7: 115-123.
54. Winzeler B, Sailer CO, Coynel D et.al. A randomized controlled trial of the GLP-1 receptor agonist dulaglutide in primary polydipsia. J Clin Invest. 2021; 15; 131: e151800.
55. Niccoli G, Cin D, Scalone G, et.al. Lipoprotein (a) is related to coronary atherosclerotic burden and a vulnerable plaque phenotype in angiographically obstructive coronary artery disease. Atherosclerosis. 2016; 246: 214-20.
56. Ooi EM, Ellis KL, Barrett PHR, et.al. Lipoprotein(a) and apolipoprotein(a) isoform size: Associations with angiographic extent and severity of coronary artery disease, and carotid artery plaque. Atherosclerosis. 2018; 275: 232-238.
57. Banach M. Lipoprotein(a): the enemy that we still don't know how to defeat. Eur Heart J Open. 2023; 3: oead080.
58. Bechlioulis A, Markozannes G, Chionidi I, et.al. The effect of SGLT2 inhibitors, GLP1 agonists, and their sequential combination on cardiometabolic parameters: A randomized, prospective, intervention study. J Diabetes Complications. 2023; 37: 108436.

Table 1. Complete treatment data for the study group.

Diabetes treatment, n (%)	
Metformin	49 (98%)
Sulfonylurea	22 (44%)

Insulin	14 (28%)
SGLT2 inhibitors	10 (20%)
DPP-4 inhibitors	4 (8%)
Other treatment, n (%)	
HMG-CoA reductase inhibitor	42 (84%)
ACEI/ARB	36 (72%)
Bblokers	21 (42%)
Indapamide	12 (24%)
Fibrates	10 (20%)
Thiazide diuretics	10 (20%)
Acetylsalicylic acid	10 (20%)
Loop diuretics	8 (16%)

Abbreviations: ACEI- Angiotensin Converting Enzyme Inhibitor; ARB- angiotensin receptor blocker; DPP-4 - Dipeptidyl peptidase-4; SGLT2 - sodium-glucosium like transporter 2;

Table 2. Baseline characteristics of patients.

	Study group	Control group
Number of patients, n	50	26
Age, years	60.8	33.1
Women, n (%)	27 (54%)	13 (50%)
Men, n (%)	23 (46%)	13 (50%)
Body mass, kg	99.9	69.7
Height, cm	168.7	175.3
BMI, kg/m2	35	22.5
Overweight n(%)	12 (24%)	5 (19%)
Obese, n (%)	35 (70%)	0
WHO guidelines on physical activity, n (%)	20 (40%)	14 (54%)
Smokers, n (%)		
Active	9 (18%)	1 (4%)
Past	11 (22%)	12 (46%)
Alcohol abuse, %	0	0
Co-morbidity, n (%)		
Hypertension	40 (80%)	0
Chronic kidney diseases	9 (18%)	0
Thyroid diseases	8 (16%)	0
Heart failure	4 (8%)	0

Abbreviations: BMI- Body mass index; WHO -World Health Organization;

Table 3. Comparison of metabolic parameters in the control and study group before treatment.

	Study group before treatment	Study group before treatment	Study group before treatment
	Mean	SD	SD
BMI kg/m^2	35.02	7.10	7.10
WHR	0.97	0.06	0.06
DBP mmHg	82.36	8.56	8.56
Copeptin pg/ml	171.50	67.55	67.55
	Median	Q1	Q3
SBP mmHg	135	125	145

Glucose mg/dl	160.5	133.1	203.3
TC mg/dl	166.55	150.3	199
LDL mg/dl	83	67	105
HDL mg/dl	48.95	43.7	56.8
non-HDL mg/dl	113.7	100	155.6
TG mg/dl	167.95	110.5	207.1
Creatinine mg/dl	1.06	0.98	1.15
GFR ml/min/1.73 m^2	67.5	58	81
HbA1C %	8.75	7.6	9.6
ALT U/I	25,5	21	42
ASP U/I	28	20	40
De Ritis ratio (ALT/ASP)	0.98	0.81	1.19
GGTP U/I	37	27	53
FIB-4	1.5	1.18	1.9
PTX3 pg/ml	1266	1164	1401
MMP-9 pg/ml	299.75	252.7	387.1
Lp(a) U/I	107.55	64.4	380.9

Abbreviations: ALT—alanine transaminase; AST—aspartate transaminase; BMI – body mass index; DBP – diastolic blood pressure; FIB-4 – fibrosis-4 score ; GFR – glomerular filtration rate; GGTP—gamma-glutamyl transpeptidase; HbA1C – glycated hemoglobin; HDL – high-density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol; Lp(a) – lipoprotein (a); MMP-9 - matrix metalloproteinase-9; non-HDL – non-high-density lipoprotein cholesterol; PTX3 – pentraxin-3; Q1 – first quartile; Q3 – third quartile; SBP – systolic blood pressure; SD – standard deviation; TC- total cholesterol; TG – triglycerides; WHR – Waist/Hip Ratio;

Table 4. Effect of glucagon-like peptide-1 receptor agonists (GLP-1RA) on metabolic parameters.

	Study group before treatment	Study group before treatment	Study group before treatment
	Mean	SD	SD
BMI kg/m^2	35.02	7.10	7.10
SBP mmHg	135.36	13.72	13.72
DBP mmHg	82.36	8.56	8.56
HbA1C %	8.68	1.52	1.52
GFR ml/min/1.73 m^2	69.76	14.51	14.51
Copeptin pg/ml	171.50	67.55	67.55
	Median	Q1	Q3
WHR	0.96	0.92	1.02
Glucose mg/dl	160.5	133.1	203.3
TC mg/dl	166.55	150.3	199
LDL mg/dl	83	67	105
HDL mg/dl	48.95	43.7	56.8
non-HDL mg/dl	113.7	100	155.6
TG mg/dl	167.95	110.5	207.1
Creatinine mg/dl	1.06	0.98	1.15
ALT U/I	25,5	21	42
ASP U/I	28	20	40
De Ritis ratio (ALT/ASP)	0.98	0.81	1.19
GGTP U/I	37	27	53
FIB-4	1.5	1.18	1.9
PTX3 pg/ml	1266	1164	1401

MMP-9 pg/ml	299.75	252.7	387.1
Lp(a) U/I	107.55	64.4	380.9

Abbreviations: ALT—alanine transaminase; AST—aspartate transaminase; BMI – body mass index; DBP – diastolic blood pressure; FIB-4 – fibrosis-4 score; GFR – glomerular filtration rate; GGTP—gamma-glutamyl transpeptidase; HbA1C – glycated hemoglobin; HDL – high-density lipoprotein cholesterol; LDL – low-density lipoprotein cholesterol; Lp(a) – lipoprotein (a); MMP-9 - matrix metalloproteinase-9; non-HDL – non-high-density lipoprotein cholesterol; PTX3 – pentraxin-3; Q1 – first quartile; Q3 – third quartile; SBP – systolic blood pressure; triglycerides SD – standard deviation; TC- total cholesterol TG;WHR – Waist/Hip Ratio;

Table 5. Correlation between changes in weight and concentration of glycated hemoglobin and concentrations of proinflammatory factors.

	ΔHbA1C	ΔWeight
ΔPTX3	R = 0.013, p>0.05	R = -0.059, p>0.05
ΔMMP-9	R = -0.035, p>0.05	R = -0.047, p>0.05
ΔLp(a)	R = -0.083, p>0.05	R = -0.38, p<0.01
ΔCopeptine	R = 0.13, p>0.05	R = -0.021, p>0.05

Abbreviations: HbA1C – glycated hemoglobin; Lp(a) – lipoprotein (a); MMP-9 - matrix metalloproteinase-9; PTX3 – pentraxin-3;

Figure 1. Flowchart of the study.

Figure 2. Concentration of Copeptin in study and control group

Figure 3. Concentration of Lipoprotein(a) [Lp(a)] in study and control group

Figure 4. Concentration of matrix metalloproteinase-9 (MMP-9) in study and control group

Figure 5. Concentration of pentraxin 3 (PTX3) in study and control group

Figure 6. Concentration of Copeptin in study group before and after treatment.

Figure 7. Concentration of Lipoprotein(a) [Lp(a)] in study group before and after treatment

Figure 8. Concentration of Matrix metalloproteinase-9 (MMP-9) in study group before and after treatment.

Figure 9. Concentration of Pentraxin 3 (PTX3) in study group before and after treatment.

Short title: GLP-1 receptor agonist and atherosclerosis