

Lipid compounds contained in sea buckthorn fruits (*Hippophae rhamnoides* L.) as ligands of G protein-coupled receptors involved in maintaining glucose homeostasis

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Obesity and its associated diseases, such as type 2 diabetes, are one of the most serious health problems worldwide. In this context, researchers are exploring new antidiabetic drugs, including those targeting G protein-coupled receptors (GPCRs). Additionally, there is a need to develop dietary strategies that can alleviate symptoms related to these conditions. One potential candidate for enriching the diet in metabolic diseases is sea buckthorn (*Hippophae rhamnoides* L.). The oil from its fruits is a source of various bioactive substances, including fatty acids, carotenoids, sterols, and tocopherols. Sea buckthorn fruits are particularly rich in the cis isomer of monounsaturated palmitoleic acid (POA), also known as 9-hexadecenoic acid (16:1n-7). Preclinical and epidemiological studies suggest that POA has a beneficial impact on disturbed metabolic parameters in type 2 diabetes and obesity. On the other hand, several studies indicate a link between elevated POA levels and type 2 diabetes, exacerbated inflammation, coronary artery disease, or fatty liver. Furthermore, POA exists in two forms: cis and trans. The trans isomer is synthesized in the digestive tract of ruminants and occurs in small amounts in dairy products. There are opinions that, unlike the harmful trans isomers derived from the hydrogenation of vegetable oils, the trans isomer of palmitoleic acid has a positive effect on human health. However, many publications (even the most recent ones) lack information about which POA isomer was the subject of research. Comparative studies on the biological activities of both isomers are also lacking.

In light of controversial data regarding palmitoleic acid and the growing interest in sea buckthorn fruit oil, it becomes necessary to conduct detailed studies on the activity of both cis and trans POA isomers, as well as oils from sea buckthorn fruits. In this doctoral dissertation, the antidiabetic potential of sea buckthorn fruit oil and oleosomes from five varieties of this plant (Prozracznaja, Luczystaja, Golden Rain, Botaniczeskaja Ljubitel'skaja, and Maryna) was evaluated. The Luczystaja variety of sea buckthorn was used to prepare cold-pressed oil, CO₂ supercritical fluid-extracted oil, hexane-extracted oil, and acetone-extracted oil using the Soxhlet method. Additionally, activated carbon-purified oil and ethyl esters of sea buckthorn fatty acids were obtained from hexane-extracted oil. Oleosomes and oils underwent simulated digestion to mimic their breakdown in the gastrointestinal tract.

Through the conducted analyses, it was found that digested hexane-extracted oil using the Soxhlet method and digested activated carbon-purified oil most significantly stimulated Glucose-Stimulated Insulin Secretion (GSIS) by activating four receptors involved in glucose and lipid homeostasis (GPR40, GPR55, GPR119, and GPR120). Both the cytotoxic effects of the tested oils and their insulinotropic activity were attributed to the presence of free fatty acids (FFAs) released during in vitro digestion. This was confirmed by studying the activity of individual fatty acids present in sea buckthorn oil (at concentrations corresponding to their content in the oil) and their mixtures. Among all the fatty acids in sea buckthorn fruit oil, palmitoleic acid exhibited the highest stimulation of insulin secretion. It also interacted with all investigated GPCR receptors, with a preference for binding to GPR40, as confirmed by molecular modeling studies conducted at the Center for Molecular and Macromolecular Research of the Polish Academy of Sciences in Łódź.

Through the analysis of sea buckthorn fruit oleosomes obtained from five different varieties, researchers at the University of Warmia and Mazury identified the Golden Rain variety as the most valuable, due to its high content of POA and carotenoids. However, it is worth noting that the POA content did not significantly differ between Golden Rain (32.8 ± 0.0 g/100 g of oil) and the Luczystaja variety (31.7 ± 0.0 g/100 g of oil). Interestingly, all digested oleosomes exhibited greater cytotoxic potential against mouse MIN6 and human EndoC- β H1 pancreatic β cells compared to the undigested samples. In the next stage of the study, the ability of oleosomes to induce GSIS was investigated. Digested oleosomes obtained from sea buckthorn fruits of the Maryna variety showed the highest insulinotropic activity in mouse and human pancreatic β -cells.

Driven by the need to resolve the biological activity of POA isomers, their effects on pancreatic β -cells MIN6 and EndoC- β H1 were investigated. It was shown that tPOA exhibits lower cytotoxicity than cPOA against the pancreatic cell lines tested. POA isomers induce insulin secretion at similar levels in both mouse and human pancreatic β -cells, activating GPR40, GPR55, GPR119 and GPR120 receptors. The trans isomer can activate GPCRs through both the pathway associated with Gq and Gs protein activation, whereas the cis isomer acts only through the Gq protein. Using molecular modelling, cPOA and tPOA were shown to have the highest affinity for GPR40.

Other lipid compounds containing fatty acid residues, such as lysophosphatidylcholines (LPCs), were previously identified as agonists for GPR40, GPR55, and GPR119 receptors. However, the activity of LPCs with acyl chains derived from cPOA and tPOA as potential ligands for these GPCRs had not been determined until now. LPCs containing acyl chains of cis and trans isomers of palmitoleic acid at the sn-1 position were synthesised in the Department of Food Chemistry and Biocatalysis at the Wrocław University of Life Sciences. These compounds induced insulin secretion with similar potency, strongly activating the GPR119 receptor and initiating the Gs cascade. Through molecular modelling methods, a preferential binding of tPOA-LPC to the GPR119 receptor was discovered.

In the final stage of this study, due to the involvement of incretin hormones in stimulating insulin secretion, the impact of sea buckthorn fruit oils and POA isomers on the secretion of glucagon-like peptide 1 (GLP-1) in enteroendocrine cells of the intestine, specifically, the mouse GLUTag line and the human NCI-H716 line was investigated. Surprisingly, neither the oils nor the POA affected GLP-1 secretion in both cell lines.

In this dissertation, the effects of sea buckthorn fruit oils and the FFAs they contain on glucose and lipid metabolism were analysed. It was shown that the insulinotropic potential of the compounds studied results from the activity of FFAs acting as ligands for selected GPCR receptors present in the membrane of pancreatic β -cells. The studies performed identified the different mechanisms of insulin secretion induced by the cis and trans isomers of POA. LPCs with POA acid residues were also identified as ligands for the GPR119 receptor. The results

provide new knowledge on the activity of POA acid isomers and prove that the oils and oleosomes studied can be used as food enrichment additives in anti-diabetic therapies.