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Unlocking the Potential of Stingray (*Dasyatis sephen*) Extract: A Novel Investigation into EPA and DHA Effects on Lipid Metabolism in High-Fat Diet-Fed Wistar Rats

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ABSTRACT

Introduction: Dyslipidemia is a complex disturbance in lipid metabolism with notable implications for cardiovascular health. Considering the burden of cardiovascular disease (CVD) on global mortality, lipid regulation should be eminently prioritized. Stingray (*Dasyatis sephen*) is one of the natural resources believed to be rich in lipid-regulating compounds: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We aimed to investigate the impact of the stingray extract on the lipid metabolism of Wistar rats subjected to a high-fat diet. **Methods:** We adopted a true experimental design with three experiment groups: a negative control (standard diet and distilled water), a positive control (high-fat diet and distilled water), and a specified treatment group (high-fat diet and stingray extract). Serum samples were collected after 28 days of treatment and analyzed for HDL and ApoB levels as dyslipidemia markers. Statistical analysis included one-way ANOVA for HDL and non-parametric Kruskal-Wallis tests for ApoB levels, followed by post-hoc analysis. **Results**: The results revealed worsening HDL and ApoB levels in the positive control group compared to the negative control group following a high-fat diet consumption. In contrast, the experiment group showed improvements in HDL and ApoB levels compared to the positive control group following the administration of the extract. However, only the dynamics in HDL level are significant ($p<0.05$), while the difference in ApoB levels among all treatment groups was not statistically significant ($p>0.05$). **Conclusion:** This study highlights the potency of D. sephen extract in regulating lipid metabolism despite the complexity of lipid metabolism regulation and the challenges of the conventional reliance on HDL and ApoB as exclusive markers of cardiovascular health.

Keywords *Dasyatis sephen*, stingray, HDL, ApoB.

INTRODUCTION

Dyslipidemia, characterized by intricate disturbances in circulating lipid profiles, assumes a pivotal and multifaceted role in contemporary medical discourse, primarily attributable to its profound implications for cardiovascular health. World Health Organization (WHO) revealed that dyslipidemia is a primary contributor to the burden of CVD, which is responsible for a substantial proportion of global mortality. The etiology of dyslipidemia is a complex entanglement of multiple aspects. Both non-modifiable and modifiable factors collectively influence its pathogenesis. Non-modifiable factors include genetic predispositions, sex-based variances, and ethnic influences, while modifiable factors include dietary preferences, physical activity levels, and tobacco usage. The inevitable wave of economic development has ushered in alterations in dietary norms, characterized by a predisposition toward animal-derived fats and sedentary lifestyles. Dyslipidemia's repercussions, however, transcend the spectrum of CVD, extending their reach into the domain of other chronic non-communicable disorders. A comprehensive understanding of the intricate web of factors governing its development is paramount to formulating effective strategies for its mitigation and promoting cardiovascular well-being globally (1–4).

Metabolism is a continuous exchange of substances and energy with the environment, supporting self-renewal. It involves two main processes: anabolism and catabolism. Lipids play a crucial role in providing energy and essential fatty acids and being necessary for cellular structures like membranes. Abnormalities in lipid metabolism can lead to pathological changes in the body (5). Lipid metabolism plays a crucial role in dyslipidemia, with factors such as the immune system (6), genetic variations (7), and lifestyle (8) influencing its regulation, and understanding these mechanisms can lead to novel therapeutic approaches for treatment.

Within the intricate landscape of lipid metabolism, two key players emerge with distinct significance: high-density lipoproteins (HDL) and apolipoprotein B-100 (ApoB-100). HDL, a fundamental component of this intricate system, orchestrates the critical process known as reverse cholesterol transport (RCT). In this process, HDL synthesized by the liver, predominantly comprising Apolipoprotein A1 (ApoA1), exerts a pivotal role. HDL serves as a molecular shuttle, shuttling cholesterol from peripheral tissues back to the liver for excretion as bile acids. This action prevents the undue accumulation of cholesterol in tissues, a critical step in maintaining cholesterol homeostasis and preventing atherogenesis. On the contrasting side, ApoB-100 takes center stage in the assembly of atherogenic lipoproteins, primarily very low-density lipoproteins (VLDLs). ApoB-100, a structural protein integral to VLDLs, plays a crucial role in their formation, ensuring the packaging of cholesteryl

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esters, triglycerides, phospholipids, and other lipids. This process sets the stage for VLDLs to transport lipids throughout the body, eventually undergoing modifications to become low-density lipoproteins (LDLs). LDLs are notorious for depositing cholesterol in arterial walls, contributing to the development of atherosclerosis, a hallmark of cardiovascular disease 9. Hence, HDL protects against cholesterol accumulation in tissues through RCT, promoting cardiovascular health. In stark contrast, ApoB-100, intimately involved in atherogenic lipoprotein assembly, underscores the importance of balanced lipid metabolism, ultimately affecting the risk of cardiovascular pathology. These two entities, HDL and ApoB-100, epitomize the intricate interplay of lipids and proteins that dictate the delicate equilibrium of cardiovascular health (5).

Stingrays (*Dasyatis sephen*), the venomous fish from the Elasmobranch family and predominantly inhabiting temperate and tropical waters hold a remarkable importance in lipid metabolism. While marine organisms constitute roughly 50% of global biodiversity, stingrays are a pivotal source of compounds that could revolutionize our understanding of lipid-related processes. Their unique environment, marked by intense competition and aggression, compels them to produce particular and potent bioactive molecules. Stingrays offer a wealth of compounds with notorious serrated spines capable of delivering venom, causing excruciating pain and tissue necrosis. This venom, with its neurotoxic, cardiotoxic, fibrinogenolytic, and anticoagulant properties, contains proteins, serotoxin, vasoconstrictor peptides, and numerous yet-to-be-identified components (9). Furthermore, the liver of the *Dasyatis sephen*, a reservoir of polyunsaturated fatty acids like EPA and DHA, emerges as a crucial player in lipid metabolism. These polyunsaturated fatty acids, notably Omega-3 (ω-3) PUFAs, are renowned for their exceptional health benefits, underlining the stingray's significance as a source of compounds that could potentially transform our understanding of lipid-related processes and their impact on human health (10,11).

EPA and DHA are PUFA found in the human diet. They play a crucial role in regulating lipid metabolism. Due to their inability to be produced internally and the requirement for dietary sources, EPA and DHA are integral for human growth and development. EPA and DHA have several notable effects in human lipid metabolism. They influence the production of enzymes and regulatory factors involved in the synthesis and breakdown of fatty acids. For instance, these omega-3 fatty acids can suppress the gene expression responsible for fatty acid up-cycle and deposit in adipose tissue, potentially limiting fat accumulation.

Additionally, they have been shown to activate genes involved in fatty acid oxidation, promoting the breakdown of fats for energy production (12,13). EPA and DHA are also known for their potential to reduce inflammation within adipose tissue, thereby affecting the overall metabolic function of white adipose tissue. This anti-inflammatory activity may reduce the emergence of metabolic disorders, including obesity and diabetes. Moreover, the abundance of EPA and DHA in the diet can impact adipose tissue composition over the long term, reflecting dietary intake patterns. Although adipose tissue typically contains low levels of these fatty acids, their supplementation can lead to modest increases in tissue levels. However, it is noteworthy that these increases may not be substantial and that other factors like weight stability can influence the fatty acid composition of adipose tissue (14,15).

This study aims to assess the impact of administering stingray (*Dasyatis sephen*) extract on the lipid metabolism of male Wistar rats (*Rattus norvegicus*) subjected to a high-fat diet. We mainly focus on changes in HDL levels and blood Apolipoprotein-β concentrations. Stingrays are known to be rich sources of EPA and DHA, which are essential components of the human diet. These fatty acids play a significant role in regulating lipid metabolism, including gene expression and enzyme activity related to fatty acid synthesis, storage, and breakdown. Understanding how stingray extract influences these parameters of a high-fat diet can provide insights into potential cardiovascular health benefits.

MATERIALS AND METHODS

Study Design

This true-experimental research was conducted at the Biochemistry Laboratory within the Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia. Ethical clearance for the study was obtained from the Ethics Committee of the Faculty of Medicine, Hang Tuah University. The research adopted a post-test-only control group design, a method chosen to bolster the study's internal validity by effectively controlling for potential confounding factors. Within this causal research design framework, the hypothesis was examined through a comparative analysis between control and treatment groups.

Population and Samples

We utilized Federer's methodology to determine the sample size and employed rigorous random selection and assignment procedures. Our study recruited 24 healthy white Wistar rats (*Rattus norvegicus*) aged 10 to 12 weeks, weighing between 130 and 170 grams, with specific criteria, including clean ocular health, glossy fur, agile mobility, and regular defecation patterns. Subjects exhibiting mortality or reduced appetite during the adaptation phase were considered drop-outs.

Our research involved an intention-to-treat approach, where the Wistar rats were subjected to a high-fat diet, and we examined the impact of varying doses of *Dasyatis sephen* extract on HDL and ApoB levels. We standardized species, weight, age, and overall physical condition. Stringent control measures were enacted to manage the participants' dietary intake, cage environment, and daily care.

Preparation of study animals

Before the treatment period, Wistar rats were acclimated to the research setting for ten days. There was free access to standard food and distilled water. Each subject was contained in 40 x 30 x 15 cm confinement with adequate light and airflow and was kept away from noises and direct sunlight to allow movements and prevent stress over the length of the study.

Preparation of Dasyatis sephen

Samples of *Dasyatis sephen* were stored at -20°C for analysis. Each sample was thawed at room temperature to measure length and weight. Liver, offal (including gut and tail), and carcass were separated and stored at -20°C in sealed poly bags. Frozen livers were weighed, thawed at around 27°C, cleaned, sliced, and minced using an electric homogenizer. Homogenized samples were weighed. To extract liver oil, 50 grams of liver samples were homogenized for two minutes in a mixture of 100 mL methanol and 50 mL chloroform. After 30 more seconds of homogenization with an additional 50 mL of chloroform, the mixture was diluted with 50 mL of distilled water. The mixture was filtered using a Whatman No.42 filter paper in a Buncher funnel under vacuum suction. After rinsing with 20 mL of chloroform and transferring the filtered portion to a separatory funnel, the chloroform layer containing liver oil was separated. The separated part underwent finer filtration using Whatman No.1 filter paper under vacuum suction and another 20 mL chloroform rinse. The filtrate was passed through 3 grams of anhydrous sodium sulfate to remove moisture. We extracted fish oil by transferring the filtrate into a dry, pre-weighed round-bottom flask attached to a rotary vacuum evaporator. The chloroform was removed at 40°C. The resulting crude liver oil extract was weighed and treated with 0.02 BTH to ensure it remained moisture-free. Finally, the prepared crude liver oil was stored at -20°C for further analysis.

Preparation of high-fat diet

We formulated the dyslipidemia-inducing diet by incorporating 15% pig oil into a standard dietary regimen. Weekly diet preparation sessions were undertaken, and the diets were meticulously stored at a controlled temperature of 24°C. Subsequent diet preparations were initiated in response to diminishing diet reserves.

Experiment

The 27 Wistar rats were divided into three groups: (1) negative-control group, (2) positive-control group, and (3) experiment group. The subjects in the negative control group received a standard diet and oral distilled water twice daily for 28 days, while subjects in the positive control group were fed a high-fat diet and given oral distilled water twice daily for 28 days. Subjects in the experiment group consumed a high-fat diet and oral distilled water twice daily for 28 days, accompanied by an administration of *Dasyatis sephen* extract via a gastric tube at a daily dose of 15 mg/kg for 14 days, starting on day 15 of the experiment.

Serum Analysis

Before extraction, subjects underwent a 12-hour fast while maintaining access to water intake. After 38 days, comprising a 10-day adaptation period and a subsequent 28-day treatment phase, all subjects received intramuscular anesthesia using ketamine at a dose ranging from 40 to 80 mg/kg. We immobilized the head and extremities then injected the posterior leg injection with anesthesia via a 33-gauge syringe. Subjects were confined until reaching a comatose state, confirmed through plantar reflex assessment. Subsequently, subjects were positioned supine and dissected over the cor region using a scalpel. Serum samples, totaling 3 ml, were collected via a perpendicular heart puncture using a syringe. These samples were promptly transferred to non-anticoagulant tubes for immediate HDL and ApoBlevel analysis, with serum separation commencing approximately 1 hour after the collection at room temperature.

For HDL analysis, serum samples were subjected to a reaction with HDL precipitating reagent, leading to apoB-containing lipoproteins precipitation, namely VLDL, LDL, and LPA (lipoprotein[a]), using phosphotungstic acid. The resultant clear supernatant, enriched with HDL, obtained through serum centrifugation, served for quantifying the HDL-cholesterol level16,17. A centrifuge tube containing 1000 L of serum and 100 L of HDL precipitating agent was then incubated for 10 minutes at room temperature. We obtained a clean supernatant by centrifuging the mixture at 4000 rpm for 20 minutes. The absorbance of this supernatant, signifying the HDL-cholesterol level, was determined using spectrophotometry at a wavelength of 546 nm (16,17).

Concurrently, for ApoB analysis, a serum separator facilitated centrifugation of serum at 3450 rpm for 15 minutes, followed by the collection of the supernatant. This supernatant underwent a second centrifugation at the same speed for 10 minutes. Subsequently, ApoB levels were quantified using the ELISA method. The ApoBsolid-phase sandwich ELISA employed a pair of antibodies to measure the target concentration. Microplate wells were pre-coated with a specific monoclonal antibody for the target. These wells received samples, standards, or controls, all bound to the immobilized (capture) antibody. After introducing a second (detector) antibody, a substrate solution served as an affinity tag, interacting with the enzyme-antibody-target complex to produce a quantifiable signal. The concentration of ApoB in the specimen directly corresponded to the signal's intensity (18).

Statistical Analysis

HDL and ApoB levels were treated as continuous variables. Before conducting statistical tests, we assessed normality and homogeneity of variance. We utilized the Shapiro-Wilk test, based on sample size, to confirm data normality, employing mean, median, mode, and standard deviation. We assessed variance homogeneity using Levene's Test to ensure distribution equivalence across study groups. If p>0.05, we assumed normality and homogeneity. The comparison of three independent groups on continuous variables involved parametric statistics, accounting for data normality and homogeneity. We employed one-way ANOVA and accepted the null hypothesis if p<0.05, indicating a statistically significant main effect. Post-hoc analysis followed significant ANOVA findings, identifying significant main effects among groups. Significance (p<0.05) revealed differences between paired groups, with the LSD technique used to adjust multiple comparisons in post hoc analysis. IBM SPSS v25.0 conducted all statistical analyses. Non-parametric analysis was used for variables lacking normality and homogeneity.

RESULTS

Based on descriptive analysis, the average level of HDL and ApoB showed a similar pattern, as shown in Table 1. In both variables, the positive control group showed a worsened level of HDL and ApoB compared to the baseline in the negative control group: a decrease in HDL level and an increase in ApoB level. In reverse, the treatment group presented improvements in HDL and ApoB levels compared to the positive control group, despite the exposure to a similar high-fat diet. The improvement in the ApoB level of the treatment group even surpasses the average level of the negative control group.

In Table 1, it is presented that the normality and homogeneity of variance were proved for the HDL level, but not for the ApoB level. Thus, we can execute a parametric comparison for the HDL level analysis must be done for the ApoB level due to unmet criteria of normality and homogeneity of variance.

Analytical statistics for HDL and ApoB are are shown in Table 2. The ANOVA of the HDL level presented significant differences between

Table 1. Descriptive statistics of HDL and ApoB levels in each of the treatment groups.

Statistics	HDL			ApoB		
	E-	E+	ET	E-	E+	ET
Mean ± SD	26,88 ± 2,167	21,50 ± 2,673	25,88 ± 3,907	0,288 ± 0,229	0,450 ± 0,450	0,200 ± 0,185
Shaphiro Wilk	0,720[a]	0,152[a]	0,227[a]	0,060[a]	0,017[b]	0,000[b]
Levene's	0,366[a]			[c]		

[a]p>0,05. Normality and homogeneity of variance was proved.
[b]p<0,05. Normality and homogeneity of variance was not proved.

Table 2. Analytical statistics of HDL and ApoB levels between each of the treatment groups.

Statistics	HDL	ApoB
ANOVA	0,004	[a]
Kruskal-Wallis	[a]	0,299

[a]The test was not executed.

Table 3. Post-hoc analysis of HDL and ApoB levels between each of the treatment groups.

Groups	HDL			ApoB		
	E-	E+	ET	E-	E+	ET
E-	[c]	0,002[a]	0,513[b]	[c]	0,517[b]	0,396[b]
E+	[c]	[c]	0,008[a]	[c]	[c]	0,116[b]
ET	[c]	[c]	[c]	[c]	[c]	[c]

[a]p>0,05. The difference between compared groups was significant.
[b]p<0,05. The difference between compared groups was not significant.
[c]No analysis executed.

the treatment groups. However, although the descriptive analysis of the ApoB level showed differences between the experiment groups, the non-parametric Kruskal-Wallis test failed to show significant differences between groups in the experiment.

The analysis was further developed in a post-hoc analysis for both HDL and ApoB levels. An LSD test was applied on the parametric data, while the Mann-Whitney test was used for the non-parametric ones. These analyses are presented in Table 3. HDL data analysis illustrated significant differences between positive control, negative control, and experiment groups. On the other hand, the difference between the negative control and experiment groups was insignificant. As expected, the dynamics of the results in the HDL levels failed to be replicated in the ApoB levels analysis. All post hoc comparisons for the ApoB levels between all the treatment groups were unable to show significant differences.

DISCUSSION

Decreased HDL and an increased concentration of ApoB in the bloodstream can profoundly affect lipid metabolism and cardiovascular health. Numerous studies have explored the implications of these changes, shedding light on their impact. A greater chance of cardiovascular disease (CVD) has been linked to low HDL levels. In the reverse cholesterol transport (RCT) mechanism, HDL is renowned for playing a pivotal role which entails removing cholesterol residue from peripheral tissues and transferring it to the liver to be disposed as bile acids. Reduced HDL levels can compromise this crucial mechanism, leading to the accumulation of cholesterol in arterial walls, a hallmark of atherosclerosis, and ultimately increasing coronary artery disease (CAD) and other cardiovascular risks and complications (19,20).

Conversely, an elevated concentration of ApoB is considerably increasing the risk for atherogenic lipoprotein formation and CVD. ApoB is a structural protein integral to VLDLs and LDLs, which are central in transporting cholesterol and triglycerides throughout the body. Increased ApoB levels can lead to higher numbers of atherogenic particles, contributing to the development and progression of atherosclerosis (21,22).

The interplay between decreased HDL and increased ApoB further exacerbates cardiovascular risk. Reduced HDL impairs the efficient removal of cholesterol from tissues, while elevated ApoB leads to increased atherogenic particles. This combination creates a proatherogenic environment, heightening the susceptibility to CAD and other cardiovascular events. In conclusion, maintaining adequate HDL levels and controlling ApoB concentrations are essential for cardiovascular health. Strategies to increase HDL, such as lifestyle modifications and pharmacological interventions, can help mitigate the risk associated with decreased HDL levels (23–25). Additionally, reducing the burden of atherogenic lipoproteins and their effects on cardiovascular health by ApoB-targeting medicines may be promising (26,27).

EPA and DHA, two long-chain PUFA (LC-PUFA), have garnered substantial attention in the field of human lipid metabolism research over the past decade. These LC-PUFA, primarily derived from marine sources such as fatty fish and algae, play pivotal roles in modulating various aspects of lipid metabolism, ultimately influencing human health profoundly. One of the most prominent effects of EPA and DHA on lipid metabolism is their ability to lower circulating triglyceride levels, a phenomenon consistently observed in numerous clinical studies (28,29). A known risk factor for cardiovascular disease is high triglyceride levels, making the triglyceride-lowering properties of EPA and DHA of particular clinical significance (30). The mechanisms through which these LC-PUFA achieve this reduction in triglycerides are multifaceted. EPA and DHA inhibit hepatic triglyceride synthesis and secretion while enhancing the clearance of triglyceride-rich lipoproteins from circulation (31,32). The detailed molecular pathways underlying these activities have been recently uncovered through recent study, highlighting the role of EPA and DHA in regulating key enzymes and transporters implicated in hepatic lipid metabolism (33). In addition to their triglyceride-lowering capabilities, EPA and DHA possess potent anti-inflammatory properties that can mitigate chronic low-grade inflammation, a hallmark of metabolic syndrome and cardiovascular disorders (34). These LC-PUFAs serve as precursors to specialized pro-resolving lipid mediators, including resolvins and protectins, which actively promote the resolution of inflammation (35). By dampening inflammation, EPA and DHA maintain metabolic homeostasis, reducing the risk of insulin resistance and associated lipid disturbances (36).

EPA and DHA also profoundly influence the composition and function of cellular membranes, including those of adipocytes and hepatocytes. Incorporating these LC-PUFA into cell membranes alters membrane fluidity and permeability, affecting cellular signaling and gene expression related to lipid metabolism (37). This membrane remodeling by EPA and DHA has been shown to upregulate genes involved in fatty acid oxidation, promoting lipid catabolism and downregulating genes associated with lipogenesis, thus reducing de novo lipid synthesis (38). Such changes at the molecular level underscore the intricate and multifaceted impact of EPA and DHA on lipid metabolism. The interaction between EPA and DHA with various nuclear receptors and transcription factors further underscores their role in lipid metabolism modulation. These LC-PUFA can trigger the peroxisome proliferator-activated receptors (PPARs), particularly PPARα, which govern gene expressions in fatty acid oxidation, transport, and utilization (30). Additionally, EPA and DHA can modulate the activity of sterol-regulatory element-binding proteins (SREBPs), key regulators of lipid biosynthesis (28). Through these interactions, EPA and DHA orchestrate a fine-tuned regulation of lipid metabolism at the genetic level.

Although EPA and DHA are not synthesized de novo by the human body, they can be derived from dietary sources, notably marine ones like fatty fish and algae (29). The capacity for humans to convert alpha-linolenic acid (ALA) into EPA and DHA is limited, emphasizing the importance of dietary intake or supplementation for achieving optimal levels of these LC-PUFA (30). However, variations in individual conversion capabilities exist, with women of childbearing age generally exhibiting higher conversion rates than men. Moreover, the bioavailability of EPA and DHA (31) depends not only on dietary sources but also on factors such as diet composition, temperature, and water salinity, which can influence the EPA and DHA content in marine organisms (32).

Due to its potential involvement in determining long-term dietary intake assessment, EPA and DHA absorption into adipose tissue has attracted interest (33). Adipose tissue's fatty acid composition reflects both dietary intake and endogenous metabolism, offering a means of assessing EPA and DHA levels in the body (34). With variations based on age, sex, and dietary preferences, the amount of EPA and DHA in human subcutaneous adipose tissue tends to be minimal (35). Interestingly, according to current research, EPA and DHA might not selectively accumulate in adipose tissue but rather might be targeted via alternative oxidation pathways or storage in substitute lipid forms, such phospholipids (36,37)—the dynamics of EPA and DHA within adipose tissue warrant further investigation to elucidate their long-term metabolic implications. Understanding the impact of EPA and DHA on these crucial lipid components is vital in elucidating their role in cardiovascular health and metabolic disorders (38,39).

HDL, often referred to as "good cholesterol," plays a pivotal role in the reverse cholesterol transport (RCT) pathway, a process that removes

excess cholesterol from peripheral tissues and transports it back to the liver for excretion (40). A higher level of HDL is generally associated with a reduced risk of cardiovascular diseases. Recent research has shed light on the favorable effects of EPA and DHA on HDL metabolism. Studies have indicated that these LC-PUFA can increase HDL cholesterol levels, promoting its functionality in RCT (41). EPA and DHA have been shown to enhance the activity of critical enzymes involved in HDL metabolism, such as lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) (28,42), that results in the esterification of free cholesterol and the transfer of cholesteryl esters to other lipoproteins, ultimately contributing to HDL maturation (29).

Furthermore, through the reduction of inflammation, which can compromise HDL's protective benefits, EPA and DHA's anti-inflammatory qualities contribute to maintaining HDL functionality (30,31). These findings underscore the potential of EPA and DHA in improving HDL-mediated cholesterol transport, a mechanism closely linked to cardiovascular health. On the other hand, ApoB is a crucial component of atherogenic lipoproteins, mainly LDL and VLDL. Due to the role that these lipoproteins play in the formation of atherosclerosis, elevated levels of ApoB are closely related to an increased risk of cardiovascular disease (32,33). Recent research has unveiled intriguing insights into how EPA and DHA may influence ApoB metabolism. Clinical studies have demonstrated that consuming EPA and DHA can reduce ApoB levels, particularly in VLDL and LDL particles (34,35). These LC-PUFA have been found to attenuate ApoB synthesis in the liver, thus reducing the production of atherogenic lipoproteins (36).

Additionally, EPA and DHA enhance the clearance of ApoB-containing lipoproteins from circulation, primarily through lipoprotein lipase (LPL) activation and LDL receptors (LDLR) upregulation (37,38). These mechanisms collectively contribute to reducing ApoB-containing particles, offering a potential therapeutic approach to mitigating cardiovascular risk. It is essential to acknowledge that the specific effects of EPA and DHA on HDL and ApoB may vary among individuals, influenced by factors such as baseline lipid profiles, genetic predisposition, and dietary habits (39,40). Determining the optimal dosages and duration of EPA and DHA supplementation for modulating HDL and ApoB levels necessitates further investigation to establish precise clinical recommendations.

In this study, the significant decrease in HDL levels following the administration of D. sephen was not accompanied by a significant increase in ApoB levels. This discrepancy of significance could be assumed due to the differences between HDL and ApoB pathways in presenting the overall lipid metabolism. Recent research has raised questions about the consistency of HDL and ApoB as markers of lipid metabolism balance. One key issue is that while HDL levels have been used as a proxy for HDL functionality, this might not always be accurate. HDL functionality involves its ability to mediate RCT effectively, and recent studies have highlighted that the quality of HDL particles can vary significantly. Some HDL particles may be dysfunctional or pro-inflammatory despite seemingly normal HDL levels. Relying solely on HDL measurements may not provide a complete picture of HDL's role in lipid metabolism (43).

Conversely, ApoB levels indicate the exclusive amount of atherogenic lipoproteins in circulation, including LDL and VLDL particles. Elevated ApoB levels indicate an increased number of potentially atherogenic particles, a significant risk factor for cardiovascular diseases. However, it is essential to recognize that ApoB does not distinguish between different types of LDL particles. Recent studies have demonstrated that the size and density of LDL particles can vary, with smaller, denser LDL particles being more atherogenic than larger, less dense ones. Therefore, relying solely on ApoB measurements may not provide information about the specific composition of LDL particles (44). To address these discrepancies and improve the accuracy of lipid metabolism assessment, researchers are increasingly exploring advanced lipid profiling method, notably nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. These techniques allow for a more detailed analysis of lipoprotein subfractions and their characteristics, including particle size, density, and composition (45). By incorporating such advanced lipid profiling methods, clinicians and researchers can better understand an individual's lipid profile, which may lead to more precise risk assessments and tailored interventions.

In conclusion, this study has shed light on the intricate interplay between stingray extract, lipid metabolism, and cardiovascular health. The findings indicate a significant decrease in HDL levels after administering D. sephen extract to rats subjected to a high-fat diet. However, this decrease was not accompanied by a significant increase in ApoB levels, highlighting the complex nature of lipid metabolism regulation. While these findings offer insightful information on the possible impacts of stingray extract on particular lipid markers, it is critical to take into account the thorough portrayal of lipid metabolism. Recent research has highlighted the limitations of relying solely on HDL and ApoB as markers of lipid metabolism balance, emphasizing the need for more comprehensive lipid profiling techniques.

To better understand the mechanisms underlying the observed variations in HDL levels and their potential impacts on cardiovascular health, further investigation is recommended. Additionally, investigating the impact of stingray extract on other aspects of lipid metabolism, could provide a more holistic understanding of its effects. Incorporating advanced lipid profiling techniques into future studies may offer a more nuanced perspective on how stingray extract influences lipid metabolism, which could inform the development of targeted therapeutic approaches for individuals with dyslipidemia and cardiovascular risk factors. Overall, this research serves as starting point for further investigations into the potential benefits and limitations of stingray extract in the context of lipid metabolism and cardiovascular health. As we unravel the complexities of lipid regulation, we move closer to developing more effective strategies for promoting cardiovascular well-being on a global scale.

CONCLUSION

Dyslipidemia, a complex condition with multifaceted risk determinants, is pivotal in cardiovascular health. The purpose of this study was to determine how stingray extract, which is high in EPA and DHA, affected the lipid metabolism in rats fed a high-fat diet. Notably, while HDL levels decreased significantly following administration of the extract, ApoB levels did not show a significant increase. These findings underscore the intricate nature of lipid metabolism regulation, challenging the traditional reliance on HDL and ApoB as exclusive markers of cardiovascular health. Advanced lipid profiling techniques may provide a more comprehensive understanding of lipid profiles and their implications, paving the way for more precise cardiovascular disease management and prevention interventions.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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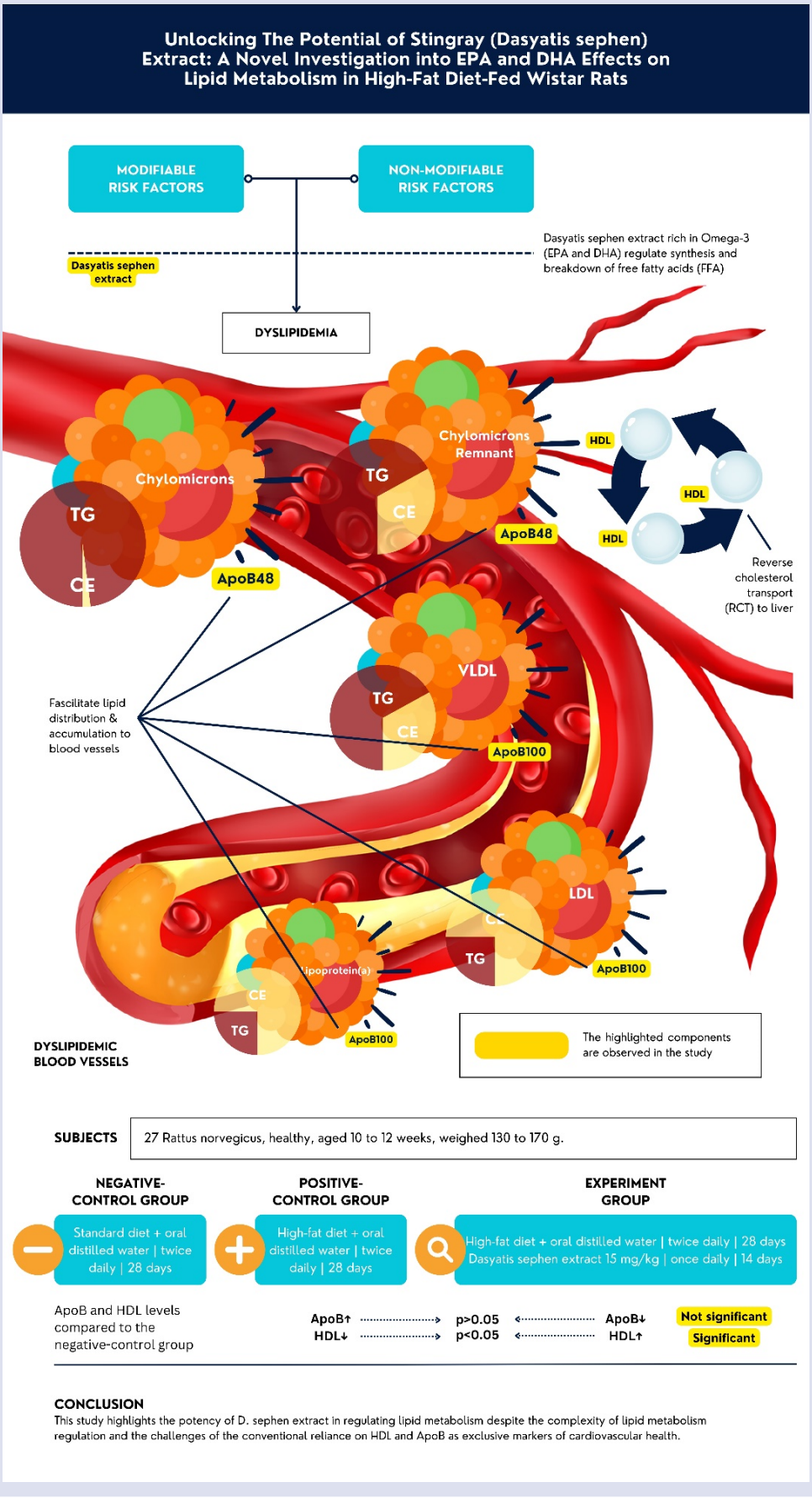
GRAPHICAL ABSTRACT



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