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Protective potential of fish oil supplementation against insulin resistance and pancreatic islet damage in STZ-induced Wistar rats

Nurina Titisari^{1,2} , Ahmad Fauzi³ , Intan Shameha Abdul Razak¹ , Nurdiana Samsulrizal⁴ 
and Hafandi Ahmad^{1,5*} 

¹Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia

²Department of Veterinary Physiology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia

³Department of Veterinary Clinical Pathology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, Indonesia

⁴Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia

⁵Institute of Tropical Agriculture and Food Safety, Universiti Putra Malaysia, Serdang, Malaysia

Abstract

Background: Fish oil, which is regarded as the primary source of omega-3 fatty acids, has been long studied for its potential as an antidiabetic therapy. However, its protective ability against insulin resistance and pancreatic islet alteration remains unclear and controversial.

Aim: To investigate the beneficial effects of fish oil consumption on the progression of insulin resistance and pancreatic islet dysfunction in a rat model of diabetes.

Methods: Diabetic rats model ($n = 30$) were divided into five groups and received; 1) NS injection + NS oral (normal control); 2) NS injection + 3 g/kg fish oil (fish oil control); 3) streptozotocin (STZ) injection + NS oral [diabetes control (DC)]; 4) STZ injection + 1 g/kg fish oil (DFO1); and 5) STZ injection + 3 g/kg fish oil (DFO3). Fasting blood insulin was analyzed by commercial rat insulin enzyme-linked immunosorbent assay; meanwhile, the determination of insulin sensitivity was calculated by homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of beta-cell function. A histological study was conducted on pancreas tissue using H and E staining.

Results: Fish oil supplementation reduced hyperglycemia and ameliorated HOMA-IR in STZ-induced animal models indicating that fish oil supplementation improved insulin sensitivity. Furthermore, animals treated with fish oil at a dose of 3 g/kg (DFO3) showed an enhancement in pancreatic islets, which was displayed by less abnormal structures than DC animals. This could imply that the administration of fish oil, especially rich in bioactive omega-3 fatty acids effectively inhibits insulin resistance and restore islet of Langerhans alteration in rats injected with STZ.

Conclusion: Thus, the current study suggested that fish oil supplementation could support the treatment of diabetes but should not be considered as an alternative therapy.

Keywords: Omega-3 fatty acids, Diabetes mellitus, Insulin, Islet of Langerhans, Wistar rats.

Introduction

Diabetes mellitus (DM) treatment still lacks an ideal drug choice to combat the disease and its associated complications (Inzucchi and McGuire, 2008; Nasri and Rafeian-Kopaei, 2014; Harrison, 2021; Fralick *et al.*, 2022). Insulin remains the primary therapy for DM, especially for type 1 DM (Malik and Taplin, 2014; Mesa, 2015). In the case of type 2 DM, lifestyle changes are required, as well as taking diabetes medication. Aside from being costly, non-insulin medication can also lead to other complications (Corathers *et al.*, 2013). Nevertheless, the use of insulin hormone therapy is

not also the ultimate solution. Health risks from having excess insulin in the blood are the development of hypertension, weight gain, and endothelial dysfunction (Kolb *et al.*, 2020). Additionally, insulin therapy must be considered, particularly in patients with congenital diseases such as heart failure (Smooke *et al.*, 2005). Therefore, it is essential to propose alternative strategies for preserving and regenerating beta-cells and preventing or delaying diabetes from its early stages (Meier, 2008; Reimann *et al.*, 2009).

The study of environmental factors contributing to the development of type 1 DM has focused on

*Corresponding Author: Hafandi Ahmad. Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Malaysia. Email: hafandi@upm.edu.my

viruses and factors that might affect the gut immune system, including hygiene and diet (Warshauer *et al.*, 2020). Diet has a substantial effect that may slow the progression of diabetes and diabetes-related complications (Asif, 2014; Khazrai *et al.*, 2014). The consumption of products rich in omega-3 fatty acids, such as plant oil, fish, and seafood, is important for diabetes management (Mali *et al.*, 2016). Many bioactive agents in marine resources, such as polyphenols, peptides, pigments, phlorotannins, and sterols, could be used to prevent and manage diabetes and its complications (Barde *et al.*, 2015). The benefits of omega-3 fatty acids supplementation in diabetes can be defined by at least two mechanisms, including anti-inflammatory activity and suppression of autoimmunity (Purdell *et al.*, 2021). Omega-3 fatty acids have been shown in numerous studies to have a beneficial impact on DM (Norris *et al.*, 2007; Li *et al.*, 2014; Iwase *et al.*, 2015; Ricordi *et al.*, 2019). In a study, fish oil from hybrid catfish was given orally to diabetic rats for 12 weeks could inhibit insulin resistance and pancreatic cell damage (Keapai *et al.*, 2016). Another study found a protective effect against progressive beta-cell damage when 8% of fish oil was added to a high fructose diet (Soltan, 2012). However, other researchers argue the health advantages and assert a lack of effects or even could increase the risk of diabetes (Poudyal *et al.*, 2011; Chauhan *et al.*, 2017; Brown *et al.*, 2019; Hu *et al.*, 2022). Therefore, a better understanding of the potential role of omega-3 fatty acids in the development and progression of diabetes sheds light on the importance of consuming omega-3 fatty acids derived from fish oil to prevent the disorder's development.

Materials and Methods

Chemicals

Streptozotocin (STZ) (Santa Cruz Biotechnology, CA, USA) was purchased from Gamma Scientific Biolab Company. The STZ solution is prepared by dissolving in sodium citrate buffer 100 mM (pH 4.5).

Experimental animals

Eight-week-old male Wistar rats ($n = 30$) weights between 250 and 280 g were used in these experiments. The Wistar rats were used in this study due to their multi-purpose characteristics in physiological and toxicological studies (Onyibe *et al.*, 2021; Edo, 2022; Edo *et al.*, 2023). The animals were acclimatized for a week and maintained in a temperature-controlled room at $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a relative humidity of $60\% \pm 10\%$. The animals were housed as three rats per cage and fed with a regular chow diet and plain water *ad libitum* until the end of the study.

Diabetes induction

After 1 week of acclimatization, diabetes induction was conducted through multiple intraperitoneal injections of 0.5 ml STZ (45 mg/kg) for 3 days to create a type 1 DM animal model. Before injection, animals were fasted for 8 hours to maximize the STZ effect. After 1

week, the blood glucose levels were estimated from the animal tail with a digital blood glucose meter (Gluco-Dr). Animals with diabetes are characterized by fasting blood glucose (FBG) exceeding 11 mMol/l (Zin *et al.*, 2019; Onyibe *et al.*, 2021).

Fish oil administration

The animal was treated with menhaden fish oil (F8020) (Sigma-Aldrich company), which contains 8%–15% docosahexaenoic acid and 10%–15% eicosapentaenoic acid. Fish oil was administered orally using oral gavage once a day, between 8 and 9 am, in a volume of 0.5 ml per rat.

Experimental design

The experimental animals were randomly allocated into five groups ($n = 6$) (Table 1). The treatment groups consist of normal control (NC), fish oil control (FC), diabetes control (DC), diabetic treated with fish oil dose 1 g/kg (DFO1), and diabetic treated with fish oil dose 3 g/kg (DFO3). Body weight (BW) was measured at the beginning and the end of the experiment. On day 7 after STZ injection, animals with FBG below 11 mMol/l were excluded from the study. Following that, depending on the group, the animals were either given normal saline or fish oil orally for the next 3 weeks. At the end of the experiment, all animals were sacrificed by an overdose of anesthesia (e.g., ketamine and xylazine) for analysis of the second FBG and pancreas tissue.

Determination of insulin sensitivity

Indirect indexes for the assessment of insulin sensitivity were calculated using fasting serum insulin and blood glucose at the end of the study. Serum insulin levels were determined using a double-antibody enzyme-linked immunosorbent assay (ELISA). Insulin ELISA kit (Rat insulin ELISA kit, BT lab) procedures followed the manufacturer's instructions. The homeostatic model assessment of insulin resistance (HOMA-IR) and the homeostatic model assessment of beta-cell function (HOMA B) index were calculated using the equation as described below (Wallace *et al.*, 2004):

$$\text{HOMA IR} = \frac{\text{Fasting peripheral insulin } (\mu\text{IU/l}) \times \text{Fasting peripheral glucose (mmol/l)}}{22.5}$$

$$\text{HOMA B} = \frac{20 \times \text{Fasting peripheral insulin } (\mu\text{IU/l})}{\text{Fasting peripheral glucose (mmol/l)} - 3.5}$$

Histological analysis

For histological analysis, the pancreas tissue of each group was fixed in phosphate buffer formalin (10%) overnight. The samples were dehydrated in graded alcohol series and cleared with xylol. Later on, dehydrated samples were embedded in paraffin and cut into sections (4 μm). The pancreas sections were stained with hematoxylin and eosin (H and E). Images were obtained with an optilab camera microscope (software image raster) using 40 \times objective lens magnification.

Statistical analysis

Statistical analysis was conducted using the standard software Statistical Package for the Social Sciences version 20.0 (SPSS, Inc., Chicago, IL) and GraphPad Prism, version 6.0 (GraphPad Software, Inc., La Jolla, CA). All data were analyzed using SPSS 20.0 software, and the data are reported as mean \pm SEM ($n = 6$ /group). Statistical analysis was performed by analysis of variance for blood glucose, BW, and insulin sensitivity, followed by the Tukey *post hoc* test to elucidate significant means. The p value ($p < 0.05$) was considered a statistical significance between groups.

Ethical approval

All animal experimental care and procedures were approved by the Institutional for Animal Care and Use Committee (IACUC), Universiti Putra Malaysia, Selangor, Malaysia (UPM/IACUC/AUP-R017/2022).

Results

The general examination in this study concluded that the diabetic classic signs were found in diabetic animals (DC, DFO1, and DFO3) which are polydipsia, polyphagia, and polyuria. Water intake of diabetic animals was higher (average 100–120 ml/rat) than control groups (NC, FC) after 7 days of STZ injection (average 50–70 ml/rat). Food intake also increased in the last 2 weeks of the experiment on diabetic animals (average 30–32 g/rat) than control animals (average 23–25 g/rat). Animal observation

also showed alterations in physical appearances and physiology between the control group and the diabetic group. Acute weight loss, decreased activity, rough coat, and diarrhea were observed in the diabetic group throughout the study.

BW and FBG levels in rats with STZ-induced diabetes before and after fish oil administration were shown in Table 2. As expected, the diabetic rats (DC, DFO1, and DFO3) exhibited increased blood glucose and decreased BW after 1 week of STZ injections. In the initial study, the FBG of diabetic groups was significantly different from NC and FC groups ($p < 0.05$), however, BW differences were not statistically significant. After 3 weeks of fish oil supplementation in diabetic animals (DFO1 and DFO3), blood glucose had returned to normal but could not prevent BW loss. This result showed a positive effect of fish oil supplementation rich in omega-3 fatty acids toward hyperglycemia inhibition in STZ-induced rats.

The NC group clearly showed significantly higher insulin levels ($p < 0.05$) compared to the diabetic groups (STZ-treated animals) (Fig. 1A). Furthermore, the HOMA-IR index, which implied body insulin resistance, was remarkably increased only in the DC group (Fig. 1B). The HOMA-B scores on DFO1 and DFO3 were significant difference ($p < 0.05$) from the NC (Fig. 1C). This means that fish oil supplementation for 3 weeks has not been able to restore beta-cells function even though the glucose level is not different from the NC group (Table 2).

As shown in Figure 2A, B, and E, Langerhans's islet appeared normal and paler than the surrounding exocrine cells. On the other hand, the islets of Langerhans of group DC and DFO1 depicted distorted pancreatic cell islets (Fig. 2C and D). Sections of the diabetic pancreas undergo shrinkage or atrophy with degeneration and necrosis of islets components cells and the disappearance of cell borders in the pancreatic islets. Nonetheless, the pathological changes in diabetic rats which received 3 g/kg/day of fish oil were less severe than those in the diabetic control group. Meanwhile, fish oil supplementation of 3 g/kg/day

Table 1. Experimental design of the treatment groups.

Group	Treatments
NC	Normal saline (NS) injection + normal saline (NS) peroral
FC	Normal saline injection + fish oil 3 g/kg
DC	STZ injection + normal saline peroral
DFO1	STZ injection + fish oil 1 g/kg
DFO3	STZ injection + fish oil 3 g/kg

The treatment groups consist of normal control (NC), fish oil control (FC), diabetes control (DC), diabetic treated with fish oil dose 1 g/kg (DFO1), and diabetic treated with Fish oil dose 3 g/kg (DFO3).

Table 2. Effect of fish oil supplementation on BW and FBG after 3 weeks of treatment.

Groups	FBG (mmol/l)		BW (g)	
	Onset of the study	End of the study	Onset of the study	End of the study
NC	5.18 \pm 0.47 ^a	5.43 \pm 0.15 ^a	253.17 \pm 9.95 ^a	319.00 \pm 16.34 ^a
FC	5.07 \pm 0.17 ^a	4.15 \pm 0.18 ^a	264.00 \pm 16.68 ^a	306.50 \pm 17.89 ^{ab}
DC	20.11 \pm 3.25 ^b	14.43 \pm 2.99 ^b	257.67 \pm 4.86 ^a	200.50 \pm 4.77 ^c
DFO1	21.18 \pm 2.04 ^b	5.53 \pm 0.71 ^a	250.00 \pm 10.38 ^a	203.83 \pm 11.85 ^c
DFO3	23.57 \pm 2.40 ^b	4.72 \pm 0.37 ^a	272.83 \pm 3.25 ^a	242.00 \pm 22.87 ^{bc}

All values are expressed as means \pm SEM ($n = 6$). Points with different superscripts in the same column show significant different ($p < 0.05$) between groups where normal control (NC), fish oil control (FC), diabetes control (DC), diabetic treated with 1 g/kg of fish oil (DFO1) and diabetic treated with 1 g/kg of fish oil (DFO3).

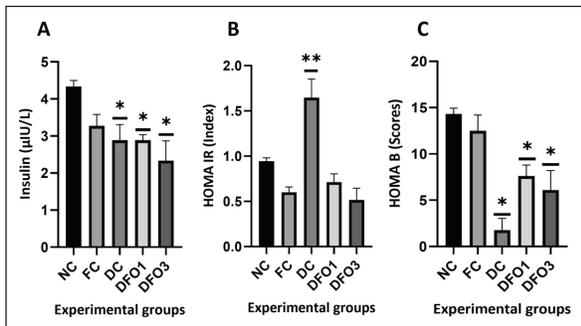


Fig. 1. Control negative group (NC) had higher insulin levels (A), the diabetic control group (DC) was insulin resistant (B), and beta-cell dysfunction (C). All values are expressed as means \pm SEM ($n = 6$). * $p < 0.05$ versus NC; ** $p < 0.01$ versus NC.

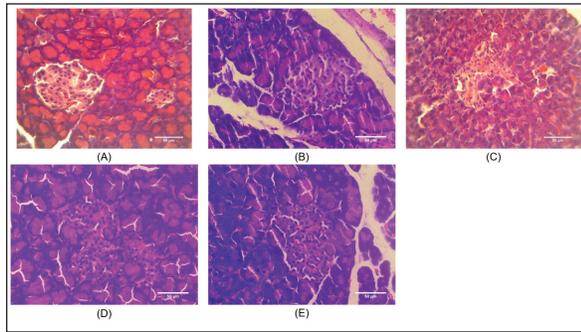


Fig. 2. Histology of treated animal pancreas tissue was as follows: (A) NC rat with a normal architecture of pancreatic islets and acinar cell; (B) FC rat (received fish oil with 3 g/kg) showed the same structure with NC rat; (C) diabetic control rat (received multiple doses of 45 mg/kg of STZ) showed distorted pancreatic islet and necrotic cells; (D) 1 g/kg of fish oil treatment on STZ-treated rat depicted islet Langerhans deformed structure but not as severe as the diabetic control group; and (E) STZ-treated rat received 3 g/kg of fish oil treatment showed slightly pathological changes on the islet of Langerhans and appear almost identical as normal group and FC group (40 \times magnification, H and E staining, scale bar: 50 μ m).

showed beneficial histological changes as it remarkably reduced pathological changes.

Discussion

Our results showed fish oil supplementation could not compromise typical symptoms of diabetes disorder in rats injected with STZ, which are conditions of excessive thirst, excessive urine, increased food consumption, and weight loss (Gyamfi *et al.*, 2019). These symptoms arise due to insulin deficiency, which causes the body to exchange proteins and fatty acids for energy sources, resulting in weight loss (Ghule *et al.*, 2010). Furthermore, high blood glucose due to insulin insufficiency initiates the kidneys to produce

more urine to eliminate the glucose from the body. This loss of fluids then causes a syndrome of polydipsia, inducing the individual to increase water intake (Bakris *et al.*, 2009; Gerich, 2010; Uduak Akpan *et al.*, 2012). Even so, in this study, the fish oil-treated STZ-induced animals showed abnormalities characteristic of diabetes even though the blood glucose reached the normal level. We suspected STZ injection causing complications in other organs besides the pancreas, such as the kidneys and liver. As it has been established that STZ binding to the glucose transporter 2 (GLUT2) in the plasma membrane then produces pathological effects on the development of diabetes in the animal model (Lenzen, 2008; Šoltésová and Herichová, 2011). Whereas other cells besides the pancreas could express GLUT2 transporters, such as renal tubular cells and hepatocytes, which then impact the organ structure and function (Eleazu *et al.*, 2013). Moreover, the complication is not scientifically proven due to limitations in this study, but some investigators reported that fish oil could not completely inhibit pathological changes in the kidneys (Ghadge *et al.*, 2016; Parveen *et al.*, 2019) or liver (Jangale *et al.*, 2013) of rats injected with STZ.

FBG levels in the diabetic control rats increased at the beginning, indicating that the STZ injection method in this study was damaging pancreatic beta-cells. Surprisingly, all diabetic rats had lower blood glucose levels by the end, yet DC rats were still considered hyperglycemia which is defined as being above 11 mMol/l (Dong *et al.*, 2014; DiMeglio *et al.*, 2018; Samsulrizal *et al.*, 2021). Meanwhile, the fish oil treatment group in this study was within normal limits (4–6 mMol/l) (Wang *et al.*, 2010). In line with previous observations, FBG was reduced after 4 weeks of fish oil supplementation in STZ-induced rats (Parveen *et al.*, 2019). These FBG results were then calculated with fasting serum insulin levels to obtain HOMA-IR and HOMA-B. It is well-known that HOMA is a non-invasive method of measuring insulin resistance and pancreatic beta-cell function with a formula derived from examining plasma glucose and insulin levels (Bellia *et al.*, 2018).

As depicted in Figure 1, serum insulin levels, HOMA-B, and HOMA-IR ratio were alleviated markedly in the DC rats compared to the NC and FC rats. The DC group had the highest HOMA-IR ratio, revealing that animals encountered insulin resistance. Considering that HOMA-IR has been proven as a reliable indicator of insulin resistance in Wistar rats (Antunes *et al.*, 2016). In this study, STZ-treated rats with fish oil supplementation significantly lowered insulin resistance, as seen from the lower HOMA-IR ratio. However, both fish oil treatments failed to improve the HOMA-B scores, which were thought to be related to pancreatic beta-cell damage. It is well known that a higher HOMA-B value indicates lower beta-cell pancreas dysfunction (Wallace *et al.*, 2004; Festa *et al.*, 2008).

The HOMA-B ratio is closely associated with lower insulin production in DFO1 and DFO3 rats. This result implies that insulin secretion was not completely recovered after 3 weeks of oral fish oil administration. In contrast, scientific studies reported that omega-3 fatty acids may improve insulin secretion (Baynes *et al.*, 2018; Zou *et al.*, 2023). In the cell culture study, fish oil can prevent pancreatic cell death and enhance insulin secretion (Wei *et al.*, 2010; Das *et al.*, 2022). Meanwhile, in *in vivo* animal model, the study was limited, and the data was inconsistent. Omega-3 fatty acids-rich safflower oil has been shown to protect Fat-1 transgenic mice from insulin deficiency, high blood sugar, and beta-cell apoptosis (Bellenger *et al.*, 2011). On the other hand, long-term fish oil dietary supplementation on healthy boars had no impact on plasma glucose and insulin sensitivity (Castellano *et al.*, 2010). Data from humans also revealed conflicting findings (Mori *et al.*, 2000; Woodman *et al.*, 2002; Hendrich, 2010), indicating that the species under study, the duration of the treatment, and the capacity to control the amount of fatty acids absorbed can all have an impact on the outcomes (Wang and Chan, 2015). Histology of pancreas tissue displays two morphological and functionally distinct components by H and E staining. The exocrine pancreas (80%–85% of the pancreas) consists of acinar cells and ductal cells, and the endocrine pancreas (less than 5% of the total pancreas) consists of the islet of Langerhans (Eurell and Frappier, 2013; Zhou and Melton, 2018). The islets of Langerhans contain five types of cells: alpha cells (α -cells), beta-cells (β -cells), delta cells (δ -cells), pancreatic polypeptide cells (PP cells), and epsilon cells (ϵ -cells) (Longnecker, 2014; El Sayed and Mukherjee, 2017). Meanwhile, to distinguish beta-cells from other cells in the islet of Langerhans requires special staining such as Gomori's aldehyde fuchsin or Mallory-Azan stain (Baskin, 2015; Hull and Baskin, 2015). As a result, the present study could only display the degeneration of pancreatic islet. The islets of Langerhans were almost entirely lost in STZ-treated rats (Fig. 2C). On the other hand, the DFO3 displays a nearly normal pancreatic islet structure compared with DFO1. This study's result supports the theory of the protective effect of omega-3 fatty acids against pancreas abnormality changes in STZ-treated rats. In fact, our research finding was in line with Bellenger *et al.* (2011) and Soltan (2012) who reported that an omega-3 fatty acids enrichment diet prevents necrotic changes and atrophy of the pancreatic islet. Indeed, omega-3 fatty acids provoke beta-cell regeneration and protect the pancreas from apoptosis (Habib, 2013; Li *et al.*, 2014). According to pancreatic islet observations, 3 weeks of fish oil supplementation in STZ-induced rats appears to be able to preserve islet structural integrity, particularly with 3 g/kg fish oil. However, the fact that insulin levels were lower in STZ-treated rats implies that beta-cells damage remains to occur, as indicated

by the lower HOMA-B ratio. On the other hand, fish oil supplementation clearly showed sharply declined blood glucose levels as well as an improvement of the HOMA-IR ratio. These findings suggest that the anti hyperglycemic ability of fish oil may be due to improved insulin sensitivity, as supported by the HOMA-IR ratio. Thus, fish oil treatment likely reduced the hyperglycemia in diabetic animal models, and consequently will protect pancreatic islets from alteration.

Conclusion

In conclusion, these studies indicated that fish oil supplementation decreased blood glucose, prevented insulin resistance, and improved pancreatic islets structure in STZ-induced diabetic rats. However, it was unable to reverse the BW loss and metabolic changes caused by STZ induction in Wistar rats. Based on the limitation of this study, we suggested further investigations regarding the long-term effect of omega-3 fatty acids in the sub-acute and high-dose toxicity studies.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Nurina Titisari, Hafandi Ahmad: Conceptualization, Methodology, Analysis, Writing-Original draft. Ahmad Fauzi: Analysis. Intan Shameha Abdul Razak, Nurdiana Samsulrizal: Supervision. All Authors read and approved the final manuscript.

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Data availability

Data are available from the corresponding author, Associate Professor Dr. Hafandi Ahmad for researchers who meet the criteria for access to confidential data.

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