Ameliorating Effects of Omega-3 Fatty Acids on Underlying Mechanisms of Type 2 Diabetes

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Authors’ contributions

This work was carried out in collaboration between both authors. Author AFQ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript under the supervision of author DMS. Manuscript was finally reviewed by author DMS. Both the authors read and approved the final manuscript.

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ABSTRACT

Aims: The current study investigated the effects of polyunsaturated Omega-3-fatty acids on underlying mechanism linked with diabetes in streptozocin induced type 2 diabetic Wistar rats.

Study Design: Experimental analytical Study.

Place and Duration of Study: The study was conducted at Isra University Hyderabad and Sindh Agricultural University, Tandojam between November 2016 and November 2018.

Methodology: Seventy-five Wistar rats were assorted to five groups (15 rats per group): negative control group A and positive control group B and experimental groups C, D and E. Rats within group B,C,D, and E were injected with streptozocin (65 mg/kg body weight) to induce diabetes. Experimental groups C, D and E received Omega-3-fatty acid supplemented food in 0.3 g, 0.4 g and 0.5 g/kg bodyweight dosage for 12 weeks, respectively.

Results: Omega-3-fatty acids treated rats showed significant decrease in blood glucose level and rise in serum insulin as compared to positive control group (p-value = 0.001). At the same time, they showed significantly increased expression of insulin gene along with transcription factors: PDX1 and NKX6.1 as compared to group A (p-value = 0.001).

Conclusion: It is concluded that O3FAs reduces insulin resistance in Streptozocin-induced diabetic Wistar rats by modulating the transcription factors essential for insulin gene expression.

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1. INTRODUCTION

There are many pathological abnormalities that have been linked to the progression of diabetes. Recently altered expression of regulatory proteins (Aka transcription factors) has been known to be affiliated with diabetes. A combination of these regulatory transcription factors works in concert to monitor the expression of insulin gene and their reduced expression leading to the development of diabetes especially Type 2 [1].

Collaborated effect of homeodomain genes, Pancreatic and duodenal homeobox gene 1 (PDX1) and homeobox factor NK 6.1 (NKX6.), have been reported to be essential for organogenesis of pancreas, specifically beta cells [2-3].

PDX1 upregulates the expression of glucose transporter 2 (GLUT2), glucokinase, insulin genes 1 and 2, PDX1 itself, homeobox factor NKX6.1 and other proteins necessary for glucose detection, insulin formation and life of β cell [4-7].

Potent natural remedial products are now under consideration to reduce the occurrence and complications of diabetes. In this regard, current research work focused on the therapeutic effect of omega 3 fatty acids (O3FAs) especially Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA).

EPA and DHA via phosphorylated AMPK can show regulatory effects on glucose homeostasis and insulin sensitivity [8]. The underlying mechanisms of biological functions of O3FAs include altered membrane fluidity and activation or inhibition of transcription factors, so reducing or enhancing the expression of certain genes [9].

Main objective of current study was to evaluate the ameliorating effects of O3FAs in type 2 diabetes by upregulating the expression of PDX1 and NKX6.1 homeodomain genes.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Animals

The current research work was carried at Isra university Hyderabad and Sindh agricultural University (SAU), Tandojam. Seventy-five male rats of Wistar strain were selected for this experimental analytical study. All experimental procedures were approved by Ethical and Research Committee of Isra university (Letter # IU/RR-10/D(M&DR)/BASAR-27/2016/1573).

Male rats weighing 200–250 g/kg body weight (bw) were selected and those who achieved blood glucose level around 250 mg/dl or more. Before proceeding with the experiment, animals were made familiar with the laboratory conditions for 7 days. Rats were kept in separate stainless steel cages at 24º-25ºC temperature (12-hour light-dark cycle). Food and water were available ad libitum.

2.2 Experimental Protocol

Rats were randomly categorized into control and experimental groups (15 rats per group). Group A was set as a negative control group which received normal diet for 12 weeks. Remaining 60 animals were divided into experimental groups B, C, D & E after induction of diabetes with Streptozocin (65 mg/kg body weight intraperitoneally), after 6-8 hour fasting and later provided with 5% dextrose water to avoid hypoglycemia. Group B was kept as diabetic positive control group. Experimental groups C, D and E were provided with Omega 3 fatty acid supplemented food in 0.3 g/kg bw, 0.4 g/kg bw and 0.5 g/kg bw for 12 weeks respectively.

2.3 Blood and Tissue Sampling

After completion of experiment, animals were anesthetized with ketamine (80 mg/kg bw). The retro-orbital puncture technique was used to collect blood samples. After collection of blood samples, rats were euthanized by cervical dislocation. Blood was collected in EDTA and plain red top bottles without anticoagulant (BD, Vactainer, USA). The pancreas of each animal was removed, rinsed with normal saline and stored in “RNA Later” solution at −80ºC for RNA isolation and “Quantitative Real-Time Reverse Transcription-Polymerase Chain Reaction” (RT-PCR) analysis.

2.4 Estimation of Biochemical Parameters

The estimation of blood glucose was done by Hexokinase procedure as previously reported
3. RESULTS

Results are shown as mean ± standard deviation (S.D) with statistically significant p value ≤ 0.05. The assessment of differences in the mean values of the parameters were tested by oneway analysis of variance (ANOVA) following least significant difference (LSD) post hoc test. The graphs were made by GraphPad Prism version 8 (GraphPad Software Inc., CA, USA).

Diabetic rats treated with O3FAs in group C, D and E showed highly significant reduction in blood glucose level 351 mg/dl, 244 mg/dl and 150 mg/dl respectively in comparison to diabetic control group B (p=0.001) Figure 1. The current study corroborates that O3FAs supplementation improved the blood glucose level in experimental groups as compared to B group (450 mg/dl). While negative control group gave the normal value of 78mg/dl.

Insulin was noted as 4.3 uIU/ml in control group A rats as compared to the experimental group B 2.0 uIU/ml, group C 2.2 uIU/ml, group D 3.0 uIU/ml and group E as 3.5 uIU/ml, respectively. Serum insulin level significantly increased to near normal by the supplementation of O3FAs in group C, D and E as compared to Positive control group B. Positive control group B showed a significant decrease in serum insulin level (P=0.001) (Figure. 2).

Concerning the gene expression, the main finding of the current study was expression of insulin gene and transcription factors after O3FAs supplementation. PDX1 expression in control group A was noted as 1 ± 0.0 in control (group A) in contrast to the experimental groups B 0.03 ± 0.07, C 4.1 ± 3.5, D 9.6 ± 3.2 and E as 16.4 ± 2.4, respectively. This shows O3FAs upregulated the expression of PDX1 (P= 0.001) as exhibited in Figure. 3. NKX6.1 expression in control group A was noted as 1 ± 0.0 in control group A as compared to the experimental group B 0.13 ± 0.2, group C 2.10 ± 0.7, group D 4.32 ± 0.4 and group E as 8.6 ± 1.2, respectively. This shows that O3FAs upregulated the expression of NKX6.1 (P=0.001) as shown in Figure. 3. Relative Insulin gene expression in control group A was noted as 1 ± 0.0 in contrast to the experimental group B 0.07 ± 0.1, group C 11.46 ± 3.2, group D 13.6 ± 1.4 and group E as 18.6 ± 2.8, respectively. This shows O3FAs upregulated the expression of insulin gene (P=0.001) as shown in Figure. 3.

4. DISCUSSION

Though lots of research has been done on the effects of O3FAs on diabetes mellitus via different molecular mechanism especially modulation of four families (peroxisome proliferator activated receptor, liver X receptors, hepatic nuclear factor-4 a and sterol regulatory
element binding proteins) of transcription factors of hepatic genes. But The present study was a first alternative experimental study carried out to observe the ameliorating action of O3FAs on underlying mechanisms of diabetes and one of them is expression of transcription factors of homeodomain family like PDX1 and NKX6.1 which has been proved via current study and the results obtained have not been reported yet by other researchers.

As per available data the work on the beneficial effects of supplementation with long chain O3FAs on expression of transcription factors PDX1 and NKX6.1 is for the first time conducted in this present study in which it is evident that O3FAs up-regulated genes of Homeodomain family important for development of functionality of pancreas. These transcription factors expressed in pancreatic islets have an important action in transactivation of insulin gene.

Figure 1: Showing blood glucose (mg/dl) in control and experimental Rats with p value 0.001

Among all TFs, dealing with the glucose-dependent transcriptional regulation of β cell of pancreatic islets, PDX1 is the most favoured in combination with the NKX6.1 [2-3].

Figure 2: Serum Insulin (uIU/ml) in control and experimental Rats with p value 0.001
PDX1 and NKX6.1 appear during the growth and maturation of pancreatic islets and later get limited to the adult β cell. For the preservation of functionality and identity of β pancreatic cells, PDX1 and NKX6.1 perform a crucial role [12-14].

However, similar results regarding blood glucose and insulin have been supported by a study [15] in which the author used different proportion of omega-3 fatty acids for 90 days and reported the significant reduction in glucose and insulin resistance similar to current study in diabetic rats. Also a recent work [16] reported the similar effects of O3FAs but through different mechanism and treatment protocol. Such ameliorating effect of O3FAs on insulin sensitivity has been reported in some other animal studies as well [17-19]. The findings of current study related to glycemic status are in line with previous study conducted by Coelho [20] who reported that administration of O3FAs for 8 weeks can improve the glycemic status of diabetic subjects. However, some studies have highlighted the effect of O3FAs on expression of genes related to lipid metabolism and inflammatory biomarkers [9,21-23].

**5. CONCLUSION AND RECOMMENDATION**

The current study concludes that administration of O3FAs significantly increased the serum insulin level to near normal in experimental rats with significant decrement in blood glucose. At the same time, it upregulated the gene expression of insulin and transcription factors PDX1 & NKX6.1. Thus, it can be recommended that O3FAs can be used as an adjuvant therapeutic agent to treat and prevent type 2 diabetes. However, further animal studies and clinical trials are needed to elaborate the associated mechanisms between O3FAs supplementation and expression of PDX1 and NKX6.1.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

All experimental procedures were approved by Ethical and Research Committee of Isra university (Letter # IU/RR-10/D(M&DR)/BASAR-27/2016/1573).

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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