Journal of Pharmaceutical Research International

31(6): 1-7, 2019; Article no.JPRI.53891
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

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.

Article Information

DOI: 10.9734/JPRI/2019/v31i630379
Editor(s):
(1) Dr. Syed A. A. Rizvi, Department of Pharmaceutical Sciences, Nova Southeastern University, USA.
Reviewers:
(1) Sandip Sen, Srikrupa Institute of Pharmaceutical Sciences, India.
(2) Mohd Jokha Bin Yahya, Hospital Sultanah Aminah, Malaysia.
(3) Ilias Migdalis, Nimts Hospital, Greece.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53891

Original Research Article

Received 01 November 2019
Accepted 08 January 2020
Published 13 January 2020

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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Keywords: Diabetes mellitus; NKX6.1; Omega-3-Fatty Acids; PDX1; streptozocin.

1. INTRODUCTION

There are many pathological abnormities 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

[10,11] on Roche/Hitachi, Cobas 311 automatic analyzer (Roche diagnostic, USA). MSD 96-well MULTI- ARRAY Mouse/Rat Insulin Kit was used to measure serum insulin.

2.5 RNA Isolation and Quantitative Real-Time PCR

Total RNA was extracted from pancreatic tissue (30 mg) using Thermo scientific GeneJet RNA Purification KIT (Catalogue #K0731) according to manufacturer's instructions. Extracted RNA was quantified and qualified using NanoDrop 1000 spectrophotometer (Thermo scientific, USA). Real time PCR and cDNA synthesis were carried out via "SuperScript™ III platinum SYBR green one-step- qRT PCR kit with ROX". Reactions were set up on ice. For multiple reactions, a master mix was prepared. PCR plate was sealed and mixed gently. Reactions were placed in a preheated real-time instrument programmed as described below:

Real time instrument was programmed to perform cDNA synthesis immediately followed by PCR amplification, as shown below.

cDNA Synthesis: 50ºC for 3 minutes hold

PCR: 95ºC for 5 minutes hold:
40 cycles consist of three steps of PCR:
Denaturation 95ºC for 15 seconds
Annealing 60ºC for 30 seconds
Elongation 40ºC, 1 minute

Relative quantitation of mRNA expression was performed with "Applied Biosystems 7500" for real-time polymerase chain reaction. Following primer sequences were used in the current research. Insulin Forward: 5'-CCA GTT GGT AGA GGG AGC AG-3; insulin reverse: 5'-CAC CTT TGT GGT CCT CAC CT-3', PDX1 Forward: 5'- GGG ACC GCT CAA GTT TGT AA 3'; PDX1 Reverse: 5'-GGC TTA ACC TAA ACG CCA CA-3', NKX6.1 Forward: 5'- GGG CTT GTT GTA ATC GTC GT-3'; NKX6.1 Reverse: 5'- ACT TGG CAG GAC CAG AGA GA-3' and 18sRNAs Forward: 5'- GTA ACC CGT TGA ACC CCA TT-3'; 18s RNA Reverse: 5'- CCA TCC AAT CGC TAG TAG CG-3'

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.

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].

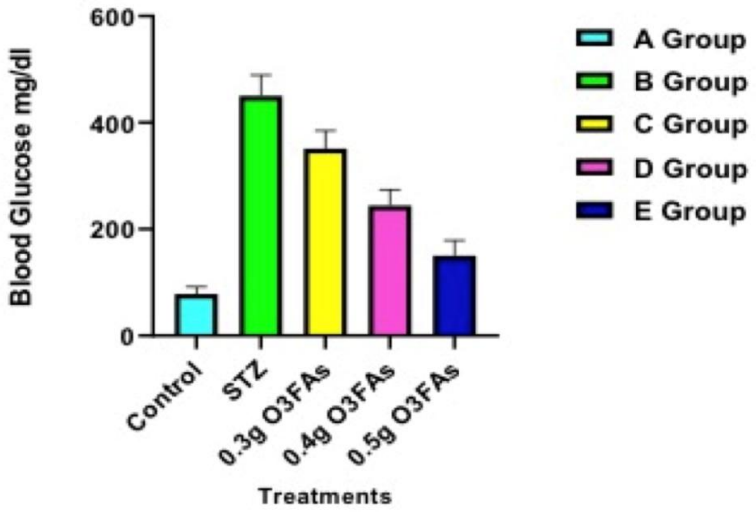

Figure 1: Showing blood glucose **(mg/dl)** in control and experimental Rats with p value 0.001

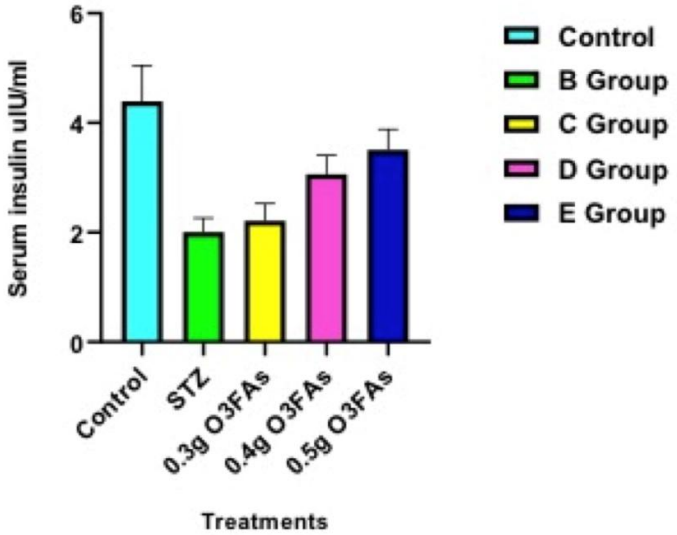

Figure 2: Serum Insulin **(uIU/ml)** in control and experimental Rats with p value 0.001

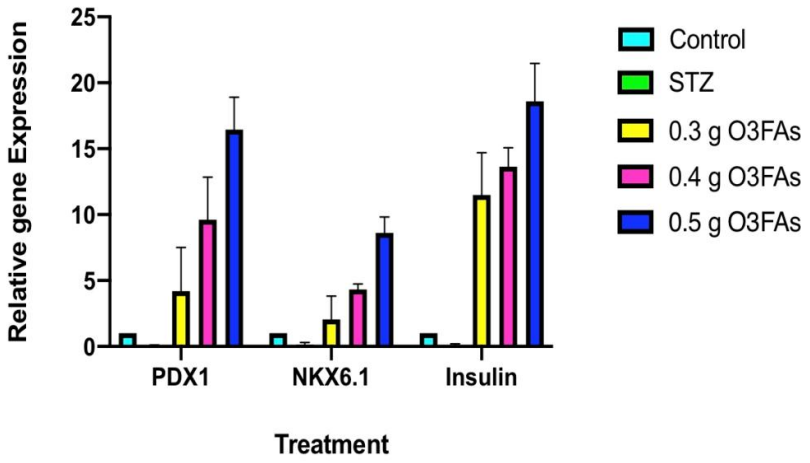

Figure 3: Relative mRNA expression of PDX1, NKX6.1 and Insulin gene in control and experimental Rats with p value 0.001

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.

REFERENCES

1. Guo S, Dai C, Guo M, Taylor B, Harmon JS, Sander M, Robertson RP, Powers AC, Stein R. Inactivation of specific β cell transcription factors in type 2 diabetes. J Clin Invest. 2013;123:3305-16.
2. Hayes HL, Moss LG, Schisler JC, Haldeman JM, Zhang Z, Rosenberg PB, Newgard CB, Hohmeier HE. Pdx-1 activates islet α-and β-cell proliferation via a mechanism regulated by transient

receptor potential cation channels 3 and 6 and extracellular signal-regulated kinases 1 and 2. Mol Cell Biol. 2013;33:4017-29.

3. Russ HA, Parent AV, Ringler JJ, Hennings TG, Nair GG, Shveygert M, Guo T, Puri S, Haataja L, Cirulli V, Blelloch R. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. The EMBO. 2015;34:1759-72.
4. Abuzgaia AM, Hardy DB, Arany E. Regulation of postnatal pancreatic Pdx1 and downstream target genes after gestational exposure to protein restriction in rats. Reproduction. 2015;149:293-303.
5. Maganti AV, Maier B, Tersey SA, Sampley ML, Mosley AL, Özcan S, Pachaiyappan B, Woster PM, Hunter CS, Stein R, Mirmira RG. Transcriptional activity of the islet β cell factor Pdx1 is augmented by lysine methylation catalyzed by the methyltransferase Set7/9. J Biol Chem. 2015;290:9812-22.
6. Malenczyk K, Szodorai E, Schnell R, Lubec G, Szabó G, Hökfelt T, Harkany T. Secretagogin protects Pdx1 from proteasomal degradation to control a transcriptional program required for β cell specification. Mol Metab. 2018;14:108-20.
7. Van der Meulen T, Huising MO. The role of transcription factors in the transdifferentiation of pancreatic islet cells. J Mol Endocrinol. 2015;54:R103.
8. Lorente-Cebrián S, Bustos M, Marti A, Martinez JA, Moreno-Aliaga MJ. Eicosapentaenoic acid stimulates AMP-activated protein kinase and increases visfatin secretion in cultured murine adipocytes. Clinl Sci. 2009;117:243-9.
9. Mazaherioun M, Saedisomeolia A, Javanbakht MH, Koohdani F, Eshraghian MR, Djalali M. Beneficial effects of n-3 polyunsaturated fatty acids on adiponectin levels and AdipoR gene expression in patients with type 2 diabetes mellitus: a randomized, placebo-controlled, double-blind clinical trial. Arch Med Sci. 2017;13: 716.
10. Kunst A, Draeger B, Ziegenhorn J. In: Bergmeyer. Methods of Enzymatic Analysis, 3rd ed. Volume VI, Metabolites 1: Carbohydrates. 1984;163-172.
11. Wu AH. Tietz clinical guide to laboratory tests-E-book. Elsevier Health Sciences; 2006.
12. Memon B, Karam M, Al-Khawaga S, Abdelalim EM. Enhanced differentiation of human pluripotent stem cells into pancreatic progenitors co-expressing PDX1 and NKX6. 1. Stem Cell Res Ther. 2018;9:15.
13. Gao T, McKenna B, Li C, Reichert M, Nguyen J, Singh T, Yang C, Pannikar A, Doliba N, Zhang T, Stoffers DA. Pdx1 maintains β cell identity and function by repressing an α cell program. Cell Metabol. 2014;19:259-71.
14. Aigha II, Memon B, Elsayed AK, Abdelalim EM. Differentiation of human pluripotent stem cells into two distinct NKX6. 1 populations of pancreatic progenitors. Stem Cell Res Ther. 2018;9:83.
15. Salim de Castro G, Deminice R, Cordeiro Simões-Ambrosio L, C Calder P, A Jordão A, Vannucchi H. Dietary docosahexaenoic acid and eicosapentaenoic acid influence liver triacylglycerol and insulin resistance in rats fed a high-fructose diet. Mar Drugs. 2015;13:1864-81.
16. Eraky SM, Abdel-Rahman N, Eissa LA. Modulating effects of omega-3 fatty acids and pioglitazone combination on insulin resistance through toll-like receptor 4 in type 2 diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids. 2018;136:123-9.
17. Lamping KG, Nuno DW, Coppey LJ, Holmes AJ, Hu S, Oltman CL, Norris AW, Yorek MA. Modification of high saturated fat diet with n-3 polyunsaturated fat improves glucose intolerance and vascular dysfunction. Diabetes Obes Metab. 2013; 15:144-52.
18. Matravadia S, Herbst EA, Jain SS, Mutch DM, Holloway GP. Linoleic and a-linolenic acid both prevent insulin resistance but have divergent impacts on skeletal muscle mitochondrial bioenergetics in obese Zucker rats. Am J Physiol Endocrinol Metab. 2014;E102-E114.
19. Nardi F, Lipina C, Magill D, Hassan RH, Hajduch E, Gray A, Hundal HS. Enhanced insulin sensitivity associated with provision of mono and polyunsaturated fatty acids in skeletal muscle cells involves counter modulation of PP2A. PloS one. 2014;9: e92255.
20. Coelho OG, da Silva BP, Rocha DM, Lopes LL, Alfenas RD. Polyunsaturated fatty acids and type 2 diabetes: Impact on the glycemic control mechanism. Crit Rev Food Sci Nutr. 2017;57:3614-9.

21. Allam-Ndoul B, Guénard F, Barbier O, Vohl MC. Effect of n-3 fatty acids on the expression of inflammatory genes in THP-1 macrophages. Lipids Health Dis. 2016; 15:69.
22. De Castro GS, Calder PC. Non-alcoholic fatty liver disease and its treatment with n-3 polyunsaturated fatty acids. Clin Nutr. 2018;37:37-55.
23. Zárate R, el Jaber-Vazdekis N, Tejera N, Pérez JA, Rodríguez C. Significance of long chain polyunsaturated fatty acids in human health. Clin Transl Med. 2017;6: 25.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53891