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OMEGA-3-FATTY ACIDS IMPROVE INSULIN RESISTANCE AND HYPERGLYCEMIA VIA UPREGULATION OF PDX1 AND NKX6.1 TRANSCRIPTION FACTORS.

[1]Dr Abroo Fatima Qazi, [2]Dr Din Muhammad Shaikh, [3]Komal Siddiqui, [4]Dr Navaid Kazi, [5]Dr Yar Muhammad Nizammani.

[1]Associate Professor , Department of Physiology, Isra University,Hyderabad, [2]Professor, Department of Physiology, Isra University,Hyderabad, [3]Assistant Professor, Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, [4]Professor, Department of Physiology, Isra University,Hyderabad, [5]Assistant Professor , Department of Physiology, Isra University,Hyderabad.

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Abstract:

Background: *Poly unsaturated Omega-3-Fatty Acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been widely acclaimed as having a broad spectrum of therapeutic properties. These Fatty acids have been reported to show beneficial effects in improving the diabetic pathologies like insulin resistance and its consequent dyslipidemia.*

Objectives Of Study: *To observe the health beneficial effects of long chain omega-3-fatty acids on reversing the insulin resistance and correction of its consequent dyslipidemia in Streptozocin induced diabetic rats.*

Subjects And Methods: *An experimental – analytical study was conducted at the ISRA University, Hyderabad and Sindh Agricultural University, Tandojam. 75 Male Wistar Albino rats were selected for this study and were divided into 5 groups, Control group A and experimental groups B,C,D and E, comprising of 15 rats in each group. Group A was taken as non-diabetic control group and was given normal chow diet while group B was taken as diabetic control after inducing diabetes via Streptozocin 65 mg /kg body weight. Omega-3-fatty acids were given in 03 different doses 0.3g, 0.4g and 0.5g per kg body weight to experimental groups C, D and E respectively in which diabetes was induced with Streptozocin similar to diabetic control group. Animal were handled under close supervision during 3 month's duration experiment. The blood samples and pancreatic tissue were stored and processed in a systemic way. Blood glucose and serum insulin were determined according to standard methods. HOMA-IR was calculated to assess insulin resistance. Pancreatic tissue was used RT-PCR. Collected data was analyzed on SPSS version 21.0. (IBM, corporation, USA) P-value of significance was taken at 0.05.*

Results: *Blood glucose lowering activity of O3FAs was observed at 03 different doses(0.3g, 0.4g and 0.5g). Serum insulin was improved to near normal in experimental groups C,D and E by O3FAs. The O3FAs treated animals revealed expression of major regulatory pancreatic transcription factors like PDX1 & NKX6.1 at different doses.*

Conclusion: *It is concluded that O3FAs by upregulating NKX6.1 and PDX1 genes, transactivated the insulin gene and reduced insulin resistance and dyslipidemia in STZ induced diabetic Wistar rats.*

Keywords: *Omeg-3-Fatty Acids, PDX1, NKx6.1, Insulin Resistance, Dyslipidemia.*

Corresponding author:
Dr. Abroo Fatima Qazi,
Associate Professor, Department of Physiology, Isra University, Hyderabad.

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INTRODUCTION:

The Worldwide prevalence of Diabetes Mellitus (DM) has risen dramatically over the past few decades [1]. Although prevalence of both type 1 and type 2 DM is increasing globally, the prevalence of T2DM is rising even more rapidly. Pakistan is amongst the highly affected countries and is placed at number 7 in global prevalence of DM and is expected to move to 4th place by the end of 2030 [2,3] .Insulin resistance is an early metabolic abnormality in the course of T2D [4]. Due to insulin resistance, these actions are impaired, leading to fasting hyper glycemia, elevated free fatty acids and hyperinsulinemia [5]. Insulin resistance is accepted to be a major risk factor in the etiology of type 2 diabetes mellitus, hypertension, dyslipidemia, atherosclerotic vascular disease, and may be a risk factor for coronary heart disease and stroke as well [6].

To halt the rise in T2DM, we need to look for some potential therapeutic agents that have the capability to reduce the occurrence of T2DM and may also reduce the complications associated with DM.

Poly unsaturated Omega-3-Fatty Acids (O3FAs) have been widely acclaimed as having a broad spectrum of therapeutic properties [7]. Fish and other marine animals and oils from these sources are rich in Omega 3 Fatty acids [8]. These Fatty acids have been reported to show beneficial effects in improving the diabetic pathologies like insulin resistance and its consequent dyslipidemia [9,10,11]. To halt the rise in T2DM, we need to look for some potential therapeutic agents that have the capability to reduce the occurrence of T2DM and may also reduce the complications associated with DM.

Many mechanisms have been reported through which O3FAs exert a wide range of biological actions like by affecting membrane fluidity and by modulating gene expression via regulating transcription factors related to cell differentiation and cell cycle regulation [11].

The present study will determine the health beneficial effects of long chain O3FAs on reversing the insulin resistance.

MATERIAL AND METHODS:

An experimental – analytical study was conducted at the Isra University, Hyderabad and Sindh Agricultural University, Tandojam. 75 Male Wistar Albino rats weighing 200-250 gm were selected and divided into 5: A, B,C ,D & E groups comprising of 15 rats in each group.

Prior to experimental procedure, animals were acclimatized to laboratory condition for one week. The animals were housed individually in stainless steel cages at 24°-25° C temperature with a 12-hr light-dark cycle in the animal house of Parasitology department of Sindh Agricultural University (SAU). Food and water were available *ad libitum*. All rats weighed accurately to 200-250 g and randomly divide into control and experimental groups.

All the animals were equally divided into five groups as follows:
Group A: used as the control group and was fed the normal diet for 90 days. N=15.
Group B: Diabetic control group was injected with Streptozocin (65mg/kg body weight) for documentation of diabetogenic effect in an animal model. N=15
Group C: Diabetic group (as mentioned in Group B) was fed with 0.3 g/kg body weight of Omega-3-Fatty acids for 30 days. N=15.
Group D: Diabetic rats (as mentioned in Group B) were treated with Omega 3 fatty acids with a dose of 0.4g/kg body weight. N=15
Group E: Diabetic Rats were given Omega-3-Fatty acids with a dose of 0.5g/kg for next 30 days. N=15
Chemicals:
Streptozocin (Sigma, Aldrich) & Omega-3-Fish oil (Nature's bounty) were used in this study.

Induction of Diabetes:
On experiment Day 1, all rats in experimental groups B, C, D & E were fasted for 6-8 hours prior to Streptozocin (STZ) injection. Then i.p injection of STZ dissolved in 0.9 % Saline was given at a dose of 65mg/kg body weight and then rats were given 5% dextrose water orally for next 12 hours to avoid severe hypoglycemia.

Biochemical Analysis:
Collection of blood and tissues:
On Day1 and Day 03, after fasting for 6-8 hrs, fasting blood glucose level was determined by using Breuer Glucometer to confirm diabetes and to check fasting glucose in control and experimental groups. Animals with blood glucose level 250mg/dl or above were selected for this study. Then on 90th Day of experiment, animals were anesthetized at the end of the experiment, and blood was withdrawn by the retro-orbital puncture technique. After the withdrawal of blood, the animals were sacrificed by the cervical dislocation and their pancreas were removed. Their pancreas were stored at −80 °C for future analysis.

Blood samples were collected for biochemical analysis of blood glucose & serum insulin. Sections of Pancreatic tissue were taken for RNA isolation and Reverse transcriptase polymerase chain reaction (RT-PCR) for expression of genes for insulin receptor. Insulin resistance (IR) was assessed by the homeostasis model assessment (HOMA) that is a mathematical model describing the degree of IR from fasting plasma glucose. and insulin. HOMA-estimated IR was calculated by multiplying fasting plasma insulin (mU/L) with fasting plasma glucose (mg/dl) divided by 405 [12].

The total RNA was extracted by using Thermo scientific GeneJet RNA Purification KIT f. Ribosomal RNA 18s was used as an internal control

RESULTS:
Collected data was analyzed on SPSS version 21.0. (IBM, corporation, USA) P-value of significance was taken at 0.05.

In the current study, Streptozocin induced diabetic rats in group C,D, and E were treated with three different doses of O3FAs. It was found that serum insulin level was normalized in dose-dependent manner. It was also observed that all three doses ,0.3, 0.4 and 0.5 g of O3FA in group C, D & E respectively showed a significant decrease in blood glucose and serum insulin compared with Experimental Group B.

The primary outcome variables of study were an increase in insulin gene expression level and decrease in homeostasis model assessment-estimated insulin resistance (HOMA-IR) by Omega 3 Fatty acids (O3FA).

Groups	Mean	F Value	P value
A	1 ± 0.00	125.24	0.001
B	0.03 ± 0.07		
C	4.19 ± 3.35		
D	9.6 ± 3.2		
E	16.44 ± 2.4		

Table 1:mRNA expression of *PDX1* in Control and Experimental Groups (n=75)

Groups	Mean	F value	P value
A	1 ±.00	183.3	0.001
B	0.15 ± 0.21		
C	2.45 ± 1.72		
D	1.6 ± 0.4		
E	8.6 ± 1.2		

Table 2: mRNA expression of *NKX6.1* in Control and Experimental Groups (n=75)

Groups	Means	F value	P value
A	1 ± 0.00	239.8	0.001
B	0.07± 0.12		
C	11.46 ± 3.2		
D	13.64 ± 1.42		
E	18.60 ± 2.87		

Table 3: mRNA expression of Insulin in Control and Experimental Groups (n=75)

Groups	Glucose	Insulin	HOMA-IR	P value
A	78 ± 13.33	4.3 ± 0.6	0.89 ± 0.27	0.001
B	450 ± 39.21	2.0 ± 0.2	2.2 ± 0.3	
C	351 ± 33.3	2.2 ± 0.3	1.9 ± 0.3	
D	244 ± 30.4	3.0 ± 0.3	1.8 ± 0.2	
E	150 ± 28.7	3.5 ± 0.4	1.2 ± 0.4	

Table 4: Glucose (mg/dl), Serum Insulin (uIU/ml) and HOMA-IR (%) **in Control and Experimental Groups (n=75)**

DISCUSSION:

A number of medications have been developed over time to combat DM, but still the quality of life remains unpleasant due to type 2 DM. This necessitates a further investigation for the treatment of type 2 DM to help come up with alternative synthetic drugs like herbal fish oil that can alleviate various physical grievances that are accompanied by DM. The present study was an alternative experimental study conducted to observe the ameliorating effects of O3FAs on insulin resistance and dyslipidemia in Streptozocin induced diabetic rat model.

Poly-unsaturated fatty acid (PUFA), predominantly comprising eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA), has been broadly used in daily life. Several sources of evidence indicate that PUFA is capable of improving lipid disorders as well as of ameliorating insulin resistance.

Recently, the beneficial effect of PUFA on insulin sensitivity has been demonstrated in animal studies [13,14]. Some studies have investigated the effect of PUFA on blood glucose level and insulin in diabetic adults [15,16, 17,18,19].

Hyperglycemia, insulin resistance, oxidative stress, low intensity chronic inflammation and dyslipidemia are the main complications related to diabetes. Improved insulin resistance and glycemic control are essential targets for t type 2 diabetics treatment [18]. According to some authors, O3FAs consumption may be beneficial on glycemic control [15,18,19] and insulin sensitivity improvement [19].

However, to the best of our knowledge, this study is the first interventional trial revealing the beneficial effects of supplementation with long chain O3FAs on expression of transcription factors PDX1 and NKX6.1, important for insulin gene expression, in Beta cells of

Pancreas in a rat model with T2DM. O3FAs influenced expression of several genes central to cell metabolism. Our study results have showed that O3FAs upregulated genes of Homeodomain family important for development of pancreas functionality, such as NKX6.1 and PDX1.

During the pancreatic islet cell development, Transcription factors, play an integral role in directing cell fates by regulating the transcriptional network controlling pancreatic specification and ultimately mature function. Several members of the homeodomain family of transcription factors, including Pdx1, Nkx2.2, Nkx6.1and Pax4, are involved in the development of the various pancreatic islet cell types (α, β, and δ) and maintenance of their differentiated functions (Bansal, V.S.,*et* al,2013) [20]. Among all transcription factors involved the glucose-dependent transcriptional control of beta cell of pancreatic islets, PDX1 is most favoured one and in combination with the Nkx6.1 (Russ, H.A., *et al*,2015; Hayes, H.L., *et al*,2013) [21,22].

REFERENCE:

1. Bano, G., Ali, S.A., Mawani, M. and Ali, S.A., 2016. Burden of Diabetes Mellitus and Role of Telemedicine in its Management: Narrative Review. *Annals of Clinical and Laboratory Research.*, *4*(3), p.113.
2. Akhtar, S., Khan, Z., Rafiq, M. and Khan, A., 2016. Prevalence of Type II diabetes in District Dir Lower in Pakistan. *Pakistan journal of medical sciences*, *32*(3), p.622.
3. Ansari, R.M., Hosseinzadeh, H. and Zwar, N.A., 2016. Primary healthcare system of Pakistan: challenges to self-management of type 2 diabetes.
4. Mogoş, T., Dondoi, C., Chelan, C.V., Iacobini, A. and Buzea, M., 2014. Effect of Omega-3 Fatty Acids Treatment on Insulin Resistance. *Romanian Journal of Diabetes Nutrition and Metabolic Diseases*, *21*(4), pp.269-276.
5. Lalia, A. and Lanza, I., 2016. Insulin-sensitizing effects of omega-3 fatty acids: lost in translation?. *Nutrients*, *8*(6), p.329.
6. Imamura, F., Mukamal, K.J., Meigs, J.B., Luchsinger, J.A., Ix, J.H., Siscovick, D.S. and Mozaffarian, D., 2013. Risk factors for type 2 diabetes mellitus preceded by β-cell dysfunction, insulin resistance, or both in older adults: the Cardiovascular Health Study. *American journal of epidemiology*, *177*(12), pp.1418-1429.
7. Lottenberg, A.M., da Silva Afonso, M., Lavrador, M.S.F., Machado, R.M. and Nakandakare, E.R., 2012. The role of dietary fatty acids in the pathology of metabolic syndrome. *The Journal of nutritional biochemistry*, *23*(9), pp.1027-1040.
8. El-Gendy, A.A. and Abbas, A.M., 2014. Effect of omega-3 fatty acids on haemostatic functions in urocortin-treated obese rats. *Journal of physiology and biochemistry*, *70*(3), pp.809-820.
9. Castro, G. S. F. D., Santos, R. A. D., Portari, G. V., Jordao, A. A. & Vannucchi, H. 2011. Omega 3 improves glucose tolerance but increases lipid peroxidation and DNA damage in hepatocytes of Fructose fed rats. *Applied Physiology, Nutrition,and Metabolism*, 37(2)**,** pp.233-240.
10. Bhullar, A.S., Putman, C.T. and Mazurak, V.C., 2016. Potential role of omega-3 fatty acids on the myogenic program of satellite cells. *Nutrition and metabolic insights*, *9*, pp.NMI-S27481.
11. Mazaherioun, M., Saedisomeolia, A., Javanbakht, M.H., Koohdani, F., Eshraghian, M.R. and Djalali, M., 2017. 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. *Archives of medical science: AMS*, *13*(4), p.716.
12. Eissa, L.A., Abdel-Rahman, N. and Eraky, S.M., 2015. Effects of omega-3 fatty acids and pioglitazone combination on insulin resistance through fibroblast growth factor 21 in type 2 diabetes mellitus. *Egyptian journal of basic and applied sciences*, *2*(2), pp.75-86.
13. Lamping, K.G., Nuno, D.W., Coppey, L.J., Holmes, A.J., Hu, S., Oltman, C.L., Norris, A.W. and Yorek, M.A., 2013. Modification of high saturated fat diet with n-3 polyunsaturated fat improves glucose intolerance and vascular dysfunction. *Diabetes, Obesity and Metabolism*, *15*(2), pp.144-152.
14. Matravadia, S., Herbst, E.A., Jain, S.S., Mutch, D.M. and Holloway, G.P., 2014. Linoleic and α-linolenic acid both prevent insulin resistance but have divergent impacts on skeletal muscle mitochondrial bioenergetics in obese Zucker rats. *American Journal of Physiology-Heart and Circulatory Physiology*.
15. El-Sayed, M.I.K., 2011. Effects of Portulaca oleracea L. seeds in treatment of type-2 diabetes mellitus patients as adjunctive and alternative therapy. *Journal of ethnopharmacology*, *137*(1), pp.643-651.
16. Coelho, O.G.L., da Silva, B.P., Rocha, D.M.U.P., Lopes, L.L. and Alfenas, R.D.C.G., 2017. Polyunsaturated fatty acids and type 2 diabetes: impact on the glycemic control mechanism. *Critical reviews in food science and nutrition*, *57*(17), pp.3614-3619.

17. Müllner, E., Plasser, E., Brath, H., Waldschütz, W., Forster, E., Kundi, M. and Wagner, K.H., 2014. Impact of polyunsaturated vegetable oils on adiponectin levels, glycaemia and blood lipids in individuals with type 2 diabetes: a randomised, double-blind intervention study. *Journal of human nutrition and dietetics*, *27*(5), pp.468-478.
18. Ogawa, S., Abe, T., Nako, K., Okamura, M., Senda, M., Sakamoto, T., Ito, S. and DIMS Study Group, 2013. Eicosapentaenoic acid improves glycemic control in elderly bedridden patients with type 2 diabetes. *The Tohoku journal of experimental medicine*, *231*(1), pp.63-74.
19. Sarbolouki, S., Javanbakht, M.H., Derakhshanian, H., Hosseinzadeh, P., Zareei, M., Hashemi, S.B., Dorosty, A.R., Eshraghian, M.R. and Djalali, M., 2013. Eicosapentaenoic acid improves insulin sensitivity and blood sugar in overweight type 2 diabetes mellitus patients: a double-blind randomised clinical trial. *Singapore Med J*, *54*(7), pp.387-390.
20. Bansal, V.S., Raja, C.P., Venkataraman, K. and Vijayalakshmi, M.A., 2013. Genes involved in pancreatic islet cell rejuvenation. *The Indian journal of medical research*, *137*(4), p.695.
21. Russ, H.A., Parent, A.V., Ringler, J.J., Hennings, T.G., Nair, G.G., Shveygert, M., Guo, T., Puri, S., Haataja, L., Cirulli, V. and Blelloch, R., 2015. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *The EMBO journal*, *34*(13), pp.1759-1772.
22. Hayes, H.L., Moss, L.G., Schisler, J.C., Haldeman, J.M., Zhang, Z., Rosenberg, P.B., Newgard, C.B. and Hohmeier, H.E., 2013. 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. *Molecular and cellular biology*, *33*(20), pp.4017-4029.