EFFECT OF NICOTINIC ACID ON TRIACYLGLYCEROL LEVELS IN DIABETIC AND NON-DIABETIC RATS

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ABSTRACT

Back Ground: Determination of nicotinic acid effectiveness on serum triacylglycerol levels in diabeticandnon-diabeticrats

Methods: It was an experimental study conducted at the Institute of Molecular Biology and Biotechnology at the University of Lahore, Pakistan. Nicotine acid was given for a total of 42 days to diabetic and non-diabetic albino rats and a treatment response was registered on day 21 and on day 42 of the experiment. The analysis of variance (Anova) was used to compare these two groups and the control groups. P value of ($p < 0.05$) was considered as significant. The research included a total of 50 rats.

Results: In comparison with control groups, serum levels of triacylglycerol (TAG) decreased significantly with nicotinic acid in the dyslipidemic group and the dyslipidemic + diabetic group on 21st and the day 42.

Conclusion: The nicotinic acid is an effective agent in lowering the TAGdyslipidemiain diabetic and nondiabetic rats.

KEY WORDS: Nicotinic acid, Dyslipidemic profile, Triacylglycerol,

INTRODUCTION

The ability of nicotinic acid to modify the lipids is well defined. The fundamental mechanism is that nicotinic acid suppresses Triglyceride hormone- sensitive lipase activity. This leads to a reduced release of adipose tissue free fatty acids. Nicotinic acid is considered a strong medicine for lipid and lipoprotein defects. Nicotinic acid can also serve as a miracle medicine to cure lipid metabolic disorders. The water-soluble vitamin is being used over many years to treat lipid diseases which, if left untreated, would lead to cardiac diseases and atherosclerosis. It is able to increase HDL cholesterol significantly. The cholesterol, lipoprotein VLDL, LDL, TAG and fatty acids are also reduced[1]. In the past use of nicotinic acid asdrug has been discontinued since nicotinic acid therapy causes extremely troubling skin flushing that cannot be tolerated by the clients. The extended use of nicotinic acid has, however, been shown to reduce the occurrence of hyperlipidemia by approximately 75%[2] Contrary to the prior opinion that the use of nicotinic acid in diabetics is not possible, there is now proof that it has only a moderate impact on glucose and hemoglobinA1c[3].

The accessible oral hypoglycemics can control even small modifications in the lipid profile. The use of atherosclerosismedication has apparently decreased coronary attacks in people with diabetic dyslipidemia[4] Nicotinic acid in a previous test showed a decrease of 90% in cardiac diseases occurrences. Researchers have therefore strongly suggested promoting the use of this highly efficient and inexpensive medicine, provided that the dose is increased progressively in order to prevent skin flushing. In order to assist nurses to maximize advantage from this significant cardio protective agent, efficient consultation should be provided[5]. There is little data about the use of this drug from Pakistan. This research is conducted to evaluate the nicotinic acid responsiveness in the setting of a

developing country such as Pakistan for dyslipidemic as well as dyslipidemia + diabetic subjects,when most of people cannot afford to purchase drug-like statins in order to regulate their lipid profile. In addition, an alternative therapy is accessible for those who do not consider statins appropriate due to their adverse effects. The studies were conducted on rodents due to the well-defined genetic structure which is very much like the human genetic scheme.

METHODOLOGY

Study was conducted for 42 days at the Center for Molecular Biology and Biotechnology, University of Lahore. The rats were separated into five distinct, ten rat classes. Initial weights of 240 to 300 grams were chosen for this experiment for albino rats (Rattisnorvagicus). Rats were provided an isocaloric (2700 cal / kg food) and isonitrogenic (21% harsh proteins) conventional laboratory food and had free use of drinking water throughout the study.

Group 1: Control A: Normal poultry diet

Group 2: Control B: Normal poultry diet with an oral dropper of 2 ml of olive oil a day.

Group 3: Diabetic C: Normalpoultry diet with an oral dropper of 2ml of olive oil a day.

Group 4: Hypercholesterolemic(D):Normal poultry diet + Nicotinic acid 8.5 mg/ 100g of body weight per day and 2 mL oral olive oil

Group 5: Hypercholesterolemicand diabetic (E): Normal poultry diet + nicotinic acid 8.5 mg/100 g of body weight per day, and oral dropper 2 ml of olive oil per day.

Dose Preparation: Upsher-Smith Laboratories, Inc. (Minneapolis, MN 55447 USA) made Niacin tablets (500 mg) were pulverized. For each rat of groups 4 and 5, the oral dose 8.5mg/100 g was calculated. It was made in a prescription of 2 ml of olive oil.[6]Blood sample was taken on day one, and serum was analyzed for baseline reading of above mentioned parameters. Nicotinic acid administration was started. The second sample was taken on the 21st dayandthird sample was collectedon day 42 for analysis. In the morning one ml of blood was collected from each rat's coccygeal vein. Within one hour after collection, the blood was centrifuged to separate serum. The sampleswere analyzed for blood sugar and serum triglycerides using enzyme kits; (HUMAN for cholesterol RANDOX forTG and Cenix for Blood sugar estimation).

Rats were fasted for 24 hours before diabetes induction which was carried out by intra-peritoneal administration of a single dose of 65 mg / kg body weight alloxan to rats to cause diabetes. 10 days after injecting alloxandiabetes was verified with an enzyme kit for glucose by spectrophotometer. Rats were considered diabetics with blood glucose levels above 150 mg / dl.[7]To induce hypercholesterolemia cholesterol powder in a dose of 1 g / Kg body weight was given for ten days orally to rats Group 4 and 5.[8]The serum cholesterol was verified on the eleventh day by using the test kit and a concentration above 150 mg / dl was considered as hypercholesterolemic. Impact of nicotinic acid on the lipid profile in diabetic and non- diabetic rodents was evaluated. In the descriptive statistics, all values were displayed as mean ± standard deviation (SD) and the statistical analysis for all information acquired was done using the multiple sample Variance Analysis (ANOVA)[9]. P value of less than 0.05 was considered as significant.

RESULTS

Total Cholesterol (TC) in group 4 reduced after 42 days from 213±3.02mg / dl significantly to 149±3.86mg / dl ($P<0.05$). It was found that TC was very significant after 42 days of therapy. Total Cholesterol in Group 5 reduced in a range of 225 ± 1.9 mg / dl to 131 ± 1.62 mg / dl in Group 4 and in Group 5 from228 ± 5.6 to128 ± 1.95 mg / dl, also highly significant as ($P<0.05$. The findings were both meaningful ($P<0.05$). Similarly, group and day interactions also

exhibited extremely important values as (P<0.05). Changes in Group 4 and 5 scores were very significant in comparison with group 1, group 2 or group 3.

Table 1: Mean serum Triglycerides (TG) in mg/dl (Descriptive statistics)

	Triglycerides 0 day	Triglycerides 21 day	Triglycerides 42 day
G1 (Control A on feed only)	132.50 ± 5.15	132.62 ± 4.33	132.89 ± 5.24
G2 (Control B on feed + olive oil)	132.85 ± 3.94	132.16 ± 4.24	130.84 ± 4.40
G3 (Diabetic C on feed + olive oil)	140.10 ± 1.97	138.94 ± 1.64	138.30 ± 1.42
G4 (hypercholesterolemic)	225.44 ± 1.94	169.22 ± 4.52	131.16 ± 1.63
G5 (hypercholesterolemic) + diabetes)	229.40 ± 5.53	166.30 ± 3.65	128.50 ± 1.96

The mean value for serum triglyceride (TG) was 132.50 ± 5.15, 132.62 ± 4.33 on days 21 and 132.89 ± 5.24 for days 42 in batch 1 (Control A feed only). No important serum changes were noted from day 0 to day 42 with Total Triglycerides (TG). In Group 2 (control B feed + Olive oil),theaverageserumtriglyceride(TG)was132.85±3.94,132.16±4.24and130.84±4.40 respectively on day 1, day 21 and day 42, respectively. No major changes were noted from day 0 to day 42 in serum triglycerides (TG). The serum triglycerides (TG) value was140.10± 1.97, 138.94 ± 1.64 ± 138.30 ± 1.42 on day zero and on day 21 and 42, respectively, for Group 3 (Diabetic C Feed + olive oil). The average triglyceride concentration (TG) scoreswere225.44 ±1.94, 169.22 ±4.52 and 131.16 ± on day zero, day 21, and day 42. Highly significant changes were notedbetweenday0and42inserumtriglycerides(TG).Similarly,229.40±40±5.53,166:30± 3.65 and 128.50 ± 1.96 were noted at Day 1, Day 21, and Day 42, respectively in group five (Hyperchlesterollemic + diabetic group). Highly significant changes were noted from 0 to 42,respectively.

Table 2:**Multiple comparison (LSD) of Serum Triglycerides (TG) level among different groups.**

DEPENDENT VARIABLE	(I) GROUPS	(J) GROUPS	MEAN DIFFERENCE (I-J)	STD. ERROR	SIG.[a]
Triglycerides (TG on Day 0)	Control A (Feed only)	(Control B) Feed + Olive oil	-.35	1.798	.845
		(Diabetic C) Feed + Olive oil	-7.60	1.798	.000
		Hypercholestrolemic	-92.94	1.798	.000
		Hypercholesterol + Diabetes	-96.90	1.798	.000
	Control B (Feed + Olive oil)	(Diabetic C) Feed + Olive oil	-7.25	1.798	.000
		Hypercholestrolemic	-92.59	1.798	.000
		Hypercholesterolemic + Diabetes	-96.55	1.798	.000
	Diabetic C (Feed + Olive oil)	Hypercholesterolemic emic	-85.34	1.798	.000
		Hypercholesterolemic + Diabetes	-89.30	1.798	.000
	Hypercholesterolemic emic	Hypercholesterolemic emic + Diabetes	-3.96	1.798	0.33
Triglycerides (TG on Day 21)	Control A (Feed only)	(Control B) Feed + Olive oil	.46	1.712	.789
		(Diabetic C) Feed + Olive oil	-6.32	1.712	.001
		Hypercholesterolemic emic	-36.60	1.712	.000
		Hypercholesterolemic + Diabetes	-33.68	1.712	.000
	Control B (Feed + Olive oil)	(Diabetic C) Feed + Olive oil	-6.78	1.712	.000
		Hypercholesterolemic emic	-37.06	1.712	.000
		Hypercholesterolemic	-34.14	1.712	.000
		+ Diabetes			
	Diabetic C (Feed + Olive oil)	Hypercholesterolemic emic			
		Hypercholesterolemic + Diabetes	-30.28	1.712	.000
	Hypercholesterolemic emic	Hypercholesterolemic + Diabetes	-27.36	1.712	.000
Triglycerides (TG	Control A (Feed only)	(Control B) Feed + Olive oil	2.92	1.712	.094
		(Diabetic C) Feed + Olive oil	2.05	1.488	.176
		Hypercholesterolemic	-5.41	1.488	.001

on Day 42)		Hypercholesterolemic + Diabetes	1.73	1.488	.252
	Control B (Feed + Olive oil)	(Diabetic C) Feed + Olive oil	4.39	1.488	.005
		Hypercholesterolemic emic	-7.46	1.488	.000
		Hypercholesterolemic + Diabetes	-.32	1.488	.830
	Diabetic C (Feed + Olive oil)	Hypercholesterolemic	2.34	1.488	.122
		Hypercholesterol + Diabetes	7.14	1.488	.000
	Hypercholesterolemic emic	Hypercholesterolemic + Diabetes	9.80	1.488	.000

Compared to two control groups with the diabetic C category, the serum triglyceride (TG mg / dl) was shown to differ significantly ($P<0.05$) in all classes on all days except for the start category 1 (Control A only) and group 2 (Control B on feed + olive oil). A comparison of serum triglycerides between the distinct treatment groups was observed. At day 0, 21 and 42, between Group 1 and Group 2, no substantial ($P>0.05$) serum triglyceride distinction (TG mg / dl) was found. On day 21 and 42 between group 4 (hypercholsterolemic) and group 5 (hypercholesterolemic + diabetic), similarly non-significant differences ($P>0.05$) were found in serum triglycerides (TG mg / dl). At day 42 between group 1 (feed Control A only) and group 4(hypercholesterolaemic) non-significant ($P>0.05$) serum triglycerides distinction was also noted (TG mg / dl); and on Day 42 between group 2 (feed Control B and Olive oil) and group 4 (hypercholesterol) no significant ($P>0.05$) triglyceride serum differences were observed.

DISCUSSION

A substantial decrease in hypercholesterolemia innon diabetic and hypercholesterolemic communities was found in descriptive statistics on mean serum concentrations (mg / dl) of TC, TG, LDL-C and TL. Although there has been a very small decline in the lipid profile of hypercholesterolemic diabetic mice, its findings are significant. These findings are in accordance with prior non-diabetic dyslipidemic studies[10] and diabetic dyslipidemic patient studies10[11]. Several trials have shown that the production of HDL-C is not increased by nicotinic acid, but that apoprotein A is inhibited selectively by the body and is degraded. In this phase, the HDL particles absorb cholesterol ester and throw apoproteinA back into the flow as a supporting protein for HDL particles. There is therefore more carrier protein for HDL binding. Consequently, for long periods and in large quantities HDL is accessible in circulation.[12] In our research, both non-diabetic and hypercholesterolemic diabetic rats have experienced a rise in HDL. In a research, nicotinic acid was given in a sample of rats, as well as in control group, 1/M dexamethasone and another one receiving dexamethasone+ Nicotinic acid. In the group provided nicotinic acid, serum HDL was higher. Serum complete cholesterol and serum LDL were reduced to even smaller levels than the control group. In one-third of dexamethasone, moderate fatty liver modifications were observed. Control group and the acid-receiving group did not demonstrate any indications of modifications in fat.[13]This was in line with the research on diabetic patients called diabolical assessment and efficacy evaluation Niaspan Trial (ADVENT).[14] Another human trial identified as: The arterial biology for the research of treatment effects of cholesterol reduction (ARBITER) has shown similaroutcomes.

A rise in HDL is very efficient in the inverse cholesterol transportation system by the use of enhanced tissue cholesterol, then esterified by the phosphitidylcholine cholesterol acyl transferase (PCAT). HDL is also highly efficient.[15]In addition, cholesterol is considered to be transported by means of an ATP binding protein (ABCG-1) transport system from macrophages in plaques into HDL. Cholesterol is transferred to the liver by HDL droplets straight or cholesterol by linking to VLDL or LDL by means of a cholesterol ester transmission

factor (CETP) and then to the liver. Cholesterol in the bile and bile salts are either separated by the liver. Some of them are reabsorbed by the enterohepatic circulation. The net outcome is a reduction in complete serumcholesterol.[16]

Data collected from this research are backed by prior findings that indicated a substantial reduction in triglyceride concentration of alcohol-made hypercholesterolemia in rats[17]. A similar finding was published in the research called Evaluation of Niaspan Efficiency Control and Assessment of Diabetes Efficiencies (ADVENT)[19]. It is a receptor combined with G-protein. The inhibitors of G protein coupling receptor are recruited from the ligand (nicotinic acid). The cyclic adenosine monophosphate (cAMP) decreases the activity of the protein Kinase A. This, in turn, results in reduced lipase activity; as a consequence, a reduction to adipocyte mobilization of triacylglyceroles (triglycerides) is prevented. As a result, substrate (fatty acids) are reduced to generate lipoprotein with very small lipid density in the body (VLDL). The productionoftriacylglycerols(triglycerides)inthe bodyalsoreducesbyinhibitingtheenzyme diacylglycerolacyltransferase 2. Therefore, the surplus of the carrier protein apoprotein B is reduced in liver as very low density lipoproteines are flowing in smaller quantities. Less flowing VLDLs transform into less flowing LDLs with low density lipoproteins. Therefore, the quantity of triglyceride in the blood is reduced as a consequence of the nicotinic acid administration, due to very low density lipoproteins containing triglycerides.[20]In the current investigation extremely substantial declines were found in the low density of lipoprotein cholesterol in both groups 4 and 5 ($P<0.05$). The results of this research correspond to the past research , The Arterial Biology for the Research of the Cholesterol Reduction Treatment Effects (ARBITER)[21]. The survey Prospective cardiovascular Munster studies (PROCAM) also showed similarcorrelation[22].

In response to the nicotinic acid administration ($P<0.05$), the overall value of lipid was significantly decreased. It was linked to the research carried out on humans in 1964[23].

The positive effects on atherosclerotic improvements attributed to repeated administration of nicotinic acid in diabetic patients with dyslipidemia overshadow the marginal and intermittent effects on blood glucose levels. Consensus committees were created to offer recommendations on the appropriate and healthy usage of nicotinic acid, including the American Diabetes Association, the American Heart Association, the National Cholesterol Education Program (NCEP) and the Adult Care Panel (ATP-III).The benefits of nicotinic acid have been studied as monotherapy or in conjunction with statins. Sugar concentrations have been tested in people with an altered lipid profile (both diabetic and non-diabetic). If nicotinic acid was administered alone at a load of less than or equal to 2500 g / day or in conjunction with statins, the sugar concentration increased by 4-5 per cent and the Hb A1c concentration increased by less than 0.3 per cent. The reforms were not significant. The adjustments were often slight, transient, and the values were restored to the values recorded at the outset of therapy. Changes in oral hypoglycemic agents may influence the slight rise observed. The occurrence of cardiovascular complications vs. placebo reduced when nicotinic acid has been administered for a long period. Patients were monitored for 9 years and treatment stopped. The cardiovascular hazards have been found to be reduced considerably. The use of nicotinic acid in the treatment of dyslipidemia is not a contraindication to diabetes mellitus. At the beginning of therapy and when the dose of nicotinic acid is increased, it is recommended to assess glucose levels. Lifestyle changes are recommended, as are other efforts to improve

blood glucose levels. In individuals with Hb A1c less than 8.0 percent the nicotinic acid therapy is started.[24,25]The main side effectisflushing. nausea, itching, diarrhea, reduced tolerance to glucose, hyperhomocysteinemia and other side effects. More hepatocellular injury, liver enzymes and cholestasis has been observed.To minimize dose-relatedhepatictoxicity,sustained nicotinic acid release formulations are more beneficial than the rapid release of nicotinic acid. In order to reduce its side effects, nicotinic acid preparation and the dosage must be controlled. Turning from a NA form to another should not be done without the guidance of a physician as it will cause significant liver damage[26]. Flushing frequency, the major harmful effect is decreased by about 75% with sustained nicotinic acid releaseformulations.

CONCLUSION

The research has demonstrated that nicotinic acid has a major effect on lipid profile in diabetic and non-diabetic rats. In diabetic and nondiabetic rats the serum triglycerides, serum cholesterol, have been decreased.

REFERENCES

1. Nagalaski A, Bryla J. Niacin in therapy. PostepyHig Med Dosw. (Online) 2007;15(61):288-302.

2. Prousky J. Double Agent Niacin. Its beneficial effect upon the lipid profile but its adverse effect upon plasma Homocysteine: A Case Report. Queen's Health Sci J. 2007;8(1):34-38.

3. Reasner C. Rational therapy of Mixed Dyslipidemia in a patient with Diabetes. Br J Diabetes Vascular Dis.2007;7(1):25-30.

4. Goldberg R, Jacobson T. Effects of Niacin on glucose control in patients with dyslipidemia. Mayo Clin Proc.2008;83(4):470-478.

5. ason CM, Doneen AL. Niacin: A critical component to the management of atherosclerosis: contemporary management of dyslipidemia to prevent, reduce or reverse atherosclerotic cardiovascular disease. J CardiovascNurs.2012;27(4):303-316.

6. Kolovou G, Salpea K, Cokkinos D. Comparison of Simvastatin and Nicotinicacid. Administration in Alcohol- Treated Wistar rats. Hellenic J Cardiol.2008;49:79-85.

7. Aewoye E, Olaleye S. Acid secretory response and electrolyte composition of gastric juice in diabetic rats. African J Biomedical Res.2007;10:145-151.

8. Reeves P, Nielsen F, Fahey G. AIN-93 purified diet for laboratory rodents: Final report of the American institute of Nutrition Ad Hoc writing committee on reformulation of the AIN-76 rodent diet. J Nutrition1993;123:1939-1951.

9. Steel RGD and Torrie JH. Principles and Procedures of Statistics, 2nd edition. McGraw Hill Book. Co. Inc; N.Y. U.S.A 1982;82-96.

10. Capuzzi D, Morgan J, Weiss R, Chitra R, Hutchinson H, Cressman M. Beneficial effects of rosuvastatin alone and in combination with extended-release niacin in patients with a combined hyperlipidemia and low high-denisty lipoprotein cholesterol levels. Am J Cardio.2003;91(11):1304-1310.

11. Ahmad S, Hossain A, Dey S, Akhtaruzzaman A, Siddika

    M. A comparative study on the effects of niacin plus atorvastatin with that offenofibrateplus atorvastatin in dyslipidemia patients. Bangladesh J

Pharmacol.2008;3:8-16.

12. Sakai T, Kamanna S, Kashyap M. Niacin, but Not Gemfibrozil, Selectively Increases Lp- A1, a cardio protective sub-fraction of HDL, in patients with lowHDL Cholesterol.ArteriosclerThrombVasc Biol. 2001;21:1783-1789.

13. Safaei N, Shomali T, Taherianfard M. Niacin Ameliorates Lipid Disturbances due to Glucocorticoid Administration in Rats. Iranian J Basic Med Sci.2011;15(4):997-1002.

14. Reasner C. Rational therapy of Mixed Dyslipidemia in a patient with Diabetes. Br J Diabetes Vasc Dis.2007;7(1):25-30.

15. Champ P, Harvey R. Cholesterol and steroid metabolism. Lippincott's illustrated Reviews. Biochemistery (4th edition) Lippincott Williams2007;4:234-236.

16. Chapman M. How does nicotinic acid modify the lipid profile? Euro Heart J. 2006;8(SupplF):F54-F59.

17. Kolovou G, Salpea K, Cokkinos D. Comparison of Simvastatin and Nicotinicacid. Administration in Alcohol- Treated Wistar rats. Hellenic J Cardiol.2008;49:79-85.

18. Grundy SM, Vega GL, McGovern ME et al. Efficacy, safety and tolerability of once – daily niacin for the treatment of dyslipidemia associated with type 2 diabetes. Results of the Assessment of Diabetes Control and Evaluation of the Efficacy of Niaspan Trial. Arch Intern Med2002;162:1568-76.

19. Tunaru S, Kero J, Schaub A, Wufka C, Blaukat A, Pfeffer K, et al. PUMA-G and HM74 are receptors for nicotinic acid and mediate its antilipolytic effects. Nat Med. 2003;9:352- 355.

20. Hernandez M, Samuel D, Cai T. Critical role of cholesterol ester transfer protein in nicotinic acid- mediated HDL-elevation in mice. Biochem Bio Phys Res. 2007;355(4):1075-1080.

21. Taylor A J, Sullen berger LE, Lee HJ, Lee JK, Grace KA: Arterial Biology for the Investigation of the treatment Effects of Reducing cholesterol (ARBITER) 2: a double – blind, placebo – controlled study of extended – release niacin on atherosclerosis progression in secondary prevention in patients treated with statins: Circulation. 2004;110:3512-3517.

22. Assmann G, Cullen P, Schulte H. The Munster Heart Study (PROCAM). Results of follow – up at eight years. Eur Heart J 1998: 19 (suppl A): A2 -A11.

23. Fitzgerald O, Heffernan A, Mulcahy R, Fennelly J, McFarlane R. Effect of nicotinic acid on Abnormal serum Lipids. Br Med J. 1964;1:157-159.

24. Goldberg R, Jacobson T.Effects of Niacin on glucose control in patients with dyslipidemia. Mayo Clin Proc.2008;83(4):470-478.

25. Krauss MR. Lipids and Lipoproteins in patients with type 2 Diabetes. DIABETES CARE. 2004;27(6):1496-1504.

26. Kolovou G, Salpea K, Cokkinos D. Comparison of Simvastatin and Nicotinic acid. Administration in Alcohol- Treated Wistar rats