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(Research Article)

Change of α - tocopherol, cholesterol, retinol and some fatty acids in pancreatic tissue of uncontrolled diabetic rats supported by resveratrol, lipoic acid and vitamin C

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Abstract

In this study, the effects of resveratrol, lipoic acid and vitamin C on some biochemical changes in the pancreas tissue of rats treated with streptozotocin (STZ) type 1 diabetes were investigated. Resveratrol (R), Lipoic acid (LP), and Vitamin C (VC) were administered intraperitoneally to diabetic -1 rats without insulin. According to our findings, it was observed that the α-Tocopherol amount decreased in diabetes and diabetes + antioxidant groups when compared with the control group ($p < 0.01$). Cholesterol levels did not differ between the groups. Palmitic acid (16: 0), palmitoleic acid (16: 1 n-7) and oleic acid (18: 1 n-9) in fatty acid composition decreased in diabetes and diabetes + antioxidant groups compared to control group. ($p < 0.05$, $p < 0.01$). Stearic acid (18: 0) was higher in diabetic groups than the control group. Linoleic acid (18: 2 n-6), Gamma-linolenic acid (18: 3 n-6), Arachidonic acid (20: 4 n-6) and docosahexaenoic acid (22: 6 n-3) were higher in diabetic groups ($p < 0.05$, $p < 0.01$). According to the results obtained, the antioxidant substances applied without insulin were insufficient in the tissues of rats with Type-1 diabetes requiring absolute insulin treatment. Also, the damaging effect of stz on the pancreas is observed when vitamin E levels decrease. Because vitamin E is an antioxidant substance that breaks down especially in the cell membrane and chain reaction in free radical reactions. The decrease in vitamin E levels can be explained by the increase in free radical levels in the tissue.

Keywords: Diabetes; Pancreas; α - Tocopherol; Cholesterol; Fatty Acids; Lipoic Acid; Resveratrol; Vitamin C

1. Introduction

Diabetes, known as diabetes mellitus, is a chronic disease that affects human life for many years and whose incidence is increasing in the world. It is a metabolic disorder that is mainly based on insulin deficiency, which is caused by the insufficiency of carbohydrate metabolism and then affects protein, lipid and even nucleic acid metabolism [1,3]. The antioxidant defense system is weakened and oxidative stress and free radical levels increase in diabetes.

Resveratrol (trans-3,4 ', 5-Trihydroxystilbene) is a phenolic compound found in most plant families and inhibits the fungi pathogens of plants and regulates plant-parasite interaction [4]. Resveratrol is produced by a limited number of plants (approximately 31 species). It is normally not present in high amounts in plants but is produced in stress situations. Resveratrol acts as a protection against infection and damage caused by exposure of plants to UV radiation [5,6].

Natural stilbenoids such as resveratrol are abundant in the vine, peanut, pine and Leguminosae family plants and have been proposed to have significant biological effects, such as protection from coronary heart disease and atherosclerosis [7]. Many studies have shown that natural stilbenoids, such as resveratrol, have potent antioxidant, anti-mutagenic, anti-inflammatory and cancer chemopreventive effects in carcinogenesis. Resveratrol plays a role in various biochemical and physiological events such as estrogenic, anti-platelet and anti-inflammatory properties [8,9].

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Resveratrol has been shown to be effective in preventing and alleviating diabetes in preliminary clinical studies. Recent experimental data suggests that resveratrol improves metabolic parameters in diabetic rats, decreases plasma glucose and triglyceride concentrations, and reduces insulinemia's effect [10, 11].

Although lipoic acid is taken by diet, it is synthesized by the cells of many organisms from bacteria to humans and belongs to the group of thiol compounds due to its disulfide structure. The fact that the reduced and oxidized forms have an antioxidant effect, their rapid absorption, dissolution in both liquid and lipid phases, and the ability to chelate make lipoic acid an ideal antioxidant property [12].

Ascorbic acid (Vitamin C) is a molecule with a strong antioxidant effect in the cell cytosol. Humans and primates do not have the enzyme L-gluconolactone oxidase so they cannot synthesize ascorbic acid as nove. These creatures have to take ascorbic acid, which is essential for themselves, with nutrients from outside. Vitamin C has therapeutic properties on scurvy, various cardiovascular disorders, cancer, cataract, and regulation of blood pressure. Deficiency is most prominently caused by a decrease in vitamin C in the serum. Smokers and patients with heart disease have been observed to be low [13].

In this study, we aimed to investigate the effects of resveratrol, lipoic acid and vitamin C on fatty acid profiles, lipophilic vitamins, and cholesterol parameters of rat pancreas that were insulin-dependent with experimental streptozotocin (STZ) diabetes without insulin injection.

2. Materials and methods

The experimental application was performed in Wistar albino rats with similar weights. The rats used in the experiment were grouped as follows: Control (C), Diabetes (D), Diabetes + R (D + R), Diabetes + LA (D + LA), and Diabet + VC (D + VC).

- Group C: The rats in this group were fed with normal nutrients and water during the experiment. During the experiment, only 0.9% saline was administered to this group.
- Group D: At the beginning of the experiment Streptozocin(STZ) substance was dissolved in 45 ml citric acid + 35 ml disodium hydrogen phosphate buffer solution to rats in this group and was administered intraperitoneally for a total of 50 mg/kg. The same amount of buffer without STZ was applied to the control group. Fasting blood sugars were measured three days after this application and antioxidant substances were administered after the rats became diabetic.
- Group D+R: Resveratrol dissolved in dimethylsulfoxide was administered intraperitoneally at 30 mg/kg for 6 weeks.
- Group D+LA: In this group of rats LA was dissolved in 0.1 M NaOH, and the pH was adjusted to 7.4 before administration. The injection was given intraperitoneally 50 mg/kg every other day for six weeks.
- Group D+VC: VC was dissolved in distilled water, and the pH was adjusted to 7.4 before application. Intraperitoneal injection of 100 mg/kg VC was performed for six weeks.

After the experimental applications, the liver tissues of the rats were decapitated per the decisions of the ethics committee and immediately frozen on dry ice. The samples were transferred to the tared Eppendorf tubes. Their weights were determined by weighing them on a precision scale and stored at -25 ºC until analysis.

2.1. Extraction of Lipids and Preparation of Fatty Acid Methyl Esters

The extraction of lipids from the tissue sample was performed by Hara and Radin [14] method using 3/2 (v / v) hexane-isopropanol mixture. Fatty acids in lipids were converted to derivatives such as methyl esters having volatile and stable structure for performing gas chromatographic analysis [15]. The resulting methyl esters were extracted with 2 × 5 ml of hexane. After the necessary procedures, the hexane phase was evaporated under a nitrogen stream and dissolved in 1 ml of hexane and the autosampler vials of the gas chromatography apparatus were prepared and prepared for instrument analysis.

2.2. Gas Chromatographic Analysis of Fatty Acid Methyl Esters

The fatty acids in the lipid extract were converted to methyl esters and then analyzed by SHIMADZU GC 17 gas chromatography. For this analysis, a Machery-Nagel (Germany) capillary column with a length of 25 m, an internal diameter of 0.25 μm and a PERMABOND film thickness of 25 microns was used. During the analysis, the column temperature was kept at 120-220 ºC and the injection temperature was 240 ºC and the detector temperature was 280

ºC. The column temperature program was adjusted from 120 ºC to 220 ºC. The temperature rises to 200 ºC 5 ºC / min. and 4 ºC / min from 200 ºC to 220 ºC. It was kept at 220 ºC for 8 minutes and the total time was determined as 35 minutes. Nitrogen gas was used as a carrier gas. The retention times of each fatty acid were determined by injecting mixtures of standard fatty acid methyl esters before analysis of the fatty acid methyl esters of the samples during the analysis. After this process, the necessary programming was done and the fatty acid methyl esters mixtures of the samples were analyzed [14].

2.3. Analysis of Cholesterol and Vitamin E Levels by HPLC

- g of tissue sample was weighed and homogenized with a homogenizer for 1 minute with 5 ml of hexane/isopropanol (60:40, v / v) mixture [13].
- Tissue homogenization was taken into 15 ml centrifuge tubes (Isolab Germany) and centrifuged at 6000xg for 10 minutes at 4 ºC and separated from the tissue pellet. The solvent of the supernatant was evaporated at 45 ºC and dissolved in 1 ml of acetonitrile/methanol mixture (50/50) into autosampler vials and analyzed on HPLC.
- In the analysis of cholesterol and ADEK vitamins, a mixture of 60% acetonitrile and 40% methanol was used as a mobile phase. The flow rate of the mobile phase at 1 minute was determined to be 1 ml/min. The column temperature was maintained at 30 ºC [15,16].
- Supelcosil LC 18 C (150 x 4.6 mm, 5 µm) column was used for analysis. The analysis was performed on a UV detector and the detection wavelength was 202 nm for cholesterol and vitamin E and 326 nm for retinol (vitamin A) [16,17].
- The amount of molecules found in the analysis was calculated as µg / g.

LC-10ADVP as a pump, SPD-10AVP as UV detector, CTO-10ASVP as column furnace, SIL-10ADVP as autosampler, DGU-14AVP as degasser (Shimadzu, Kyoto Japan) were used in the device. Calculations were performed with Class VP software (6.12 SP 5).

SPSS 10.0 software was used for statistical analysis. A comparison between experimental and control groups was performed using ANOVA and LSD tests.

3. Results

Table 1 Lipophilic Vitamins, Cholesterol of Pancreatic Tissue (µg/g).

Biochemical parameters	**C**	**D**	**D+LA**	**D+R**	**D+VC**
RTOC	11.89±2.45	7.78±1.99	6.11±	5.83±2.12	1.02±0.02
D_3	68.47±11.34	15.31±6.27 [d]	9.85±1.91 [d]	20.18±8.54 [d]	8.94±5.65 [d]
ATOC	101.11±23,32	75.92±24.89 [a]	67.64±8.20 [a]	45.50±14.57 [c]	14.10±2.73 [d]
ATOCAST	262.79±36.88	105.34±23.30	85.14±29.09	161.14±70.79	22.30±7.50
K1	55.75±8.26	7.48±3.65 [d]	5.28±1.83 [d]	23.17±10.38 [b]	26.06±8.79 [b]
CHOLESTEROL	3070.39±595.78	3441.11±217,16	4048.56±378.53	3289.23±383.31	3930.93±227.02
RETINOL	0.68±0.13	0.90±0.15	0.62±0.11	0.98±0.14	0.69±0.12

a- p<0.05 b-p<0.01 c-p<0.001 d-p<0.0001

According to our findings, it was observed that α-Tocopherol amount decreased in diabetes and diabetes + antioxidant groups and this decrease was higher in diabetic + VC group compared to the control group (p <0.01) (Figure 1). A group of substances called tocopherols that show antioxidant effects are called vitamin E in short. Although it has seven defined forms, α-tocopherol is the most common form of tocopherol. Although its effect has been known for many years, it has been very popular in recent years. α- Tocopherol is very resistant to heat and acids in spite of other forms. Other tocopherols are destroyed during heating, cooking, freezing, and processing of foods [18]. The main function of vitamin E is its antioxidant effect. This is a much more important feature than you thought. Because vitamin E is an antioxidant substance that breaks down especially in the cell membrane and chain reaction in free radical reactions. The decrease in vitamin E levels can be explained by the increase in free radical levels in the tissue. Also, the damaging effect of stz on the pancreas is observed by a decrease in vitamin E levels [19,20]. The amount of cholesterol; control, diabetes, and

diabetic + R groups were not significantly different, while diabetic + VC and diabetes + LA groups were found to increase compared to control (Figure 2).

In our study, it was observed that vitamin K-1 levels decreased in all diabetes and diabetes + antioxidant groups compared to control, and vitamin K-1 levels in D + La and D groups decreased more than D + R and D + VC. Vitamin K is necessary to maintain balance in the activation of blood coagulation and anticoagulation factors (carboxylation) in the liver. However, vitamin K is found in non-liver tissues, such as bone and vascular tissues, due to proteins. The main source of vitamin K-1 is green plants. However, the relationship between vitamin K levels and many diseases is not yet fully elucidated [21-23].

Retinol levels in pancreatic tissue were increased in D and D + R groups compared to control, whereas D + VC and D + LA groups did not differ significantly. Retinol plays an important role in vision [24-26]. Studies have shown that levels of retinol-bearing proteins in the tissues were affected particularly by poorly controlled diabetes. It was found that retinol concentration in liver tissues increased due to the decrease in plasma of rats with STZ diabetes compared to control rats [27]. Serum retinol concentration was found to be high in Type-I diabetic patients as in STZ-induced rats. However, it has been suggested that plasma vitamin A level is not affected in non-insulin dependent diabetes [27].

Table 2 Fatty Acid Composition of Pancreatic Tissue (%)

Fatty acids	C	D	D+LA	D+R	D+VC
16:0	25.66±,74	21.30±.88 [a]	21.37±.88 [a]	21.97±.82 [a]	20.56±.37 [a]
16:1 n-7	4.17±0.97	1.75±0.71 [b]	1.92±0.45 [b]	1.96±0.50 [b]	1.37±0.11 [b]
16:1 n-9	1.75±0.07	1.26±0.09	1.47±0.05	1.60±0.07	1.29±0.08
17:0	0.37±0.01	0.59±0.05	0.67±0.07	0.53±0.14	0.66±0.07
18:0	4.62±0.66	11.48±1.23 [c]	14.55±1.16 [c]	12.57±1.30 [c]	13.68±0.58 [c]
18:1 n-9	23.08±0.80	14.11±1.85 [a]	8.76±1.64 [a]	13.16±2.18 [a]	8.64±1.15 [a]
18:1 n-7	3.17±0.09	2.68±0.18 [b]	2.53±0.36 [b]	2.29±0.18 [b]	2.20±0.15 [b]
18:2 n-6	23.65±0.73	2691±0.70 [a]	26.88±0.86 [a]	28.2±0.36 [a]	27.99±0.49 [a]
18:3 n-6	1.78±0.13	2.32±0.18 [b]	2.11±0.23 [b]	2.61±0.20 [b]	2.72±0.21 [b]
18:3 n-3	1.09±0.09	0.70±0.16	0.90±0.17	1.07±0.13	0.56±0.05
20:0	0.05±0.01	0.85±0.17	1.23±0.32	1.01±0.47	0.80±0.18
20:1 n-9	0.31±0.01	0.71±0.08	0.81±0.17	0.74±0.15	0.84±0.12
20:2 n-6	0.30± 0.01	0.71±0.08	0.86±0.15	0.89±0.16	0.96±0.09
20:3 n-9	0.22±0.01	0.71±0,12 [b]	0,74±0,13 [b]	0,59±0,16 [b]	0,95±0,08 [b]
20:4 n-6	4,48±0,58	8,83±1.03 [b]	9.87±1.37 [b]	8.61±1.29 [b]	11.99±0.73 [b]
22:1 n-9	1.01±0.16	2.59±0.51	2.30±0.41	2.85±0.81	4.14±0.71
22:4	0.20±0.08	1.00±0.32	1.0±0.50	1.73±0.88	1.13±0.16
22:5 n-6	0.10±0.01	0.35±0.15 [a]	0.54±0.17 [b]	0.09±0.01 [a]	0.81±0.33 [b]
22:5 n-3	0.21±0.02	0.67±0.11 [b]	0.63±0.14 [b]	0.42±0.15 [b]	0.75±0.05 [c]
22:6 n-3	0.65±0.04	1.47±0.21 [b]	1.71±0.31 [b]	1.43±0.27 [b]	1.79±0.13 [b]

a- $p<0.05$ b-$p<0.01$ c-$p<0.001$ d-$p<0.0001$

In the analyzes carried out by gas chromatography, liver tissue; palmitic (16: 0), palmitoleic (16: 1 n-7 and n-9), stearic (18: 0), oleic (18: 1 n-9 and n-7), linoleic (18: 2 n-6)), linolenic (18: 3 n-3 and n-6), eicosatrienoic (20: 3 n-9), arachidonic (20: 4 n-6), docosatetraenoic (22: 4 n-6), docosapentaenoic (22: 5 n-6 and n-3) and docosahexaenoic (22: 6 n-3) acids.

It was found that palmitic acid (16:0) and palmitoleic acid (16:1, n-7) decreased in diabetic and diabetes + antioxidant groups in the fatty acid composition compared to the control group ($p < 0.05$, $p < 0.01$). The amount of oleic acid (18:1, n-9) was also decreased in diabetic and diabetes + antioxidant groups and this decrease was significantly higher in D + LA and D + VC groups ($p < 0.05$, $p < 0.01$). Stearic acid (18: 0), which is one of the saturated fatty acids, was increased in diabetic groups compared to the control group ($p < 0.001$). Eicosatrienoic (20:3, n-9) increased in all groups compared to the control group ($p < 0.01$). The amounts of linoleic acid (18:2, n-6), gamma-linolenic acid (18: 3, n-6), arachidonic acid (20:4, n-6), and docosahexaenoic acid (22:6, n-3) in diabetic and diabetic+antioxidant groups were higher ($p < 0.05$, $p < 0.01$).

4. Discussion

Advanced diabetes by increased oxidative stress and free radical give rise to many metabolic disfunction. According to fatty acid results, it is observed that the enzyme activities related to insulin-dependent fatty acid synthesis generally decrease. Because streptozotocin causes a lot of damage, especially in the pancreas, and inhibits the synthesis of enzymes responsible for fatty acid synthesis. Two different fatty acid metabolisms are effective in the mammalian group, including humans [28]. The first of these is palmitic, palmitoleic, stearic, oleic, eicosenoic, docosanoic and lignoceric acid and synthesized by Δ-9 desaturase metabolic pathway in the body as carbohydrate and amino acid precursors and found in tissue phospholipids and depot lipids. The second is essential fatty acid metabolism starting with linoleic (18:2, n-6) and linolenic acids (18:3, n-3) [18,28].

In our study, Linoleic acid (18: 2 n-6), Gamma-linolenic acid (18: 3 n-6), Arachidonic acid (20: 4) synthesized in the second metabolic pathway containing Δ-6 and Δ-5 desaturase enzymes and docosahexaenoic acid (22: 6 n-3) amounts were higher than the control group.

This result has been considered surprising by researchers in recent years. Because, in the studies carried out to date, there is a decrease in the amount of fatty acids in parallel with the damage suffered by the pancreas. In the study conducted by Brenner [29]; reported that streptozotocin decreased the amount of arachidonic acid in the formation of diabetes, suppressing the activation of delta-9, delta-6, and delta-5 desaturases. In another study performed by Doullet et al. [30] on the fatty acid changes observed in diabetes, they also examined some biochemical changes in the liver, heart, kidney, testis, spleen and brain tissues of rats treated with streptozotocin and found a decrease in the amount of arachidonic acid. However, in a recent study by Brenner [31], it was stated that the biosynthesis of polyunsaturated fatty acids from linoleic, α-linolenic and oleic acids is stimulated by dietary and hormonal factors in animal tissues. The enzymes involved in this biosynthesis are Δ6 and Δ5 desaturases and these two enzymes are only activated by the insulin hormone. Rimoldi et al., [32] also examined the activities of desaturase enzymes in insulin-resistant diabetes. Although stearoyl CoA desaturase 1 (SCD1) mRNA and enzyme activity decreased in the first three weeks period, it was determined that the mRNA of delta 6 desaturase enzyme was high and delta 6 and delta 5 desaturase activities remained unchanged. Although this result was unexpected, the reason was not fully understood.

5. Conclusion

Besides, according to all these data, the effects of antioxidant substances applied without insulin are insufficient in the tissues of rats with Type-1 diabetes requiring absolute insulin treatment.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors declared no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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