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Contribution of Vitamin C and α-Lipoic Acid and Their Combination on the Products Level of Desaturase Enzymes in the Lung and Muscle Tissues of Poorly Controlled Experimental Diabetic Rats

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Abstract: The aim of this research was to examine the effects of a Vitamin C (VC) and α-Lipoic Acid (LA) on the arachidonic and docosahexaenoic acids in fatty acid composition of lung and muscle tissues in poorly controlled diabetic rats. Unsaturated fatty acids are synthesized by the desaturase enzymes in the cells. Since desaturation of fatty acids pathway is inadequate in diabetes, essential and non essential fatty acid metabolism is acquired. Arachidonic acid and docosahexaenoic acids are products of Δ^6 desaturation pathway. In the study, 46 male Wistar rats were used and the animals were randomly allocated to the following groups: control group (n = 6), Diabetes group (D) (n = 10), D + VC (n = 10), D + α-LA (n = 10) and D + α-LA + VC (n = 10). Rats in D, D + VC, D + α-LA and D + α-LA + VC groups were intraperitoneal injected Streptozotocin (STZ) to formation of type-I diabetes and insulin was not used during the diabetes duration. Control group rats were injected with the buffer alone. After diabetic formation, the rats in D + VC, D + α-LA and D + α-LA + VC groups were administered VC and α-LA for 6 weeks. Following this process, the fatty acid composition of tissues was analyzed with gas chromatography equipment. The results showed that the level of oleic acid in tissues decreased in D and the other groups, when compared to control ($p<0.001$). The quantity of arachidonic acid in lung tissues was higher in the D and D + Antioxidant groups than in the control group ($p<0.001$). In addition, the level of the same fatty acid in muscle tissues was higher in the antioxidant group than in the control and D groups ($p<0.01$). The quantities of docosapentaenoic acid (22:5, n-3) and docosahexaenoic acid (22:6, n-3) were higher in all groups than in the control group ($p<0.0001$). The results of the present study confirmed that antioxidant agents have different effects on fatty acids synthesized by Δ^9, Δ^6 and Δ^5 desaturase enzymes in tissues of lung and muscle.

Key words: Diabetes, lung, muscle, vitamin C lipoic acid, essential fatty acids, arachidonic acid, docosahexaenoic acid

INTRODUCTION

Diabetes mellitus is a degenerative disease lead to morbidity, mortality in developed countries and is a heterogeneous disease, with a common phenotype of impaired glucose tolerance and depending on the basis of the management required to control glucose homeostasis (Berdanier, 2001; Emilien *et al*., 1999). Diabetes effects metabolism of many organs as like pancreas, muscle, kidney, eye in body.

Evidence from experimental animals indicates that diabetes effect the fatty acid composition on liver and muscle tissues time-dependent (Huang *et al*., 1984; Poisson *et al*., 1993; Shin *et al*., 1995: Dang *et al*., 1988). Since, muscle tissue lacks the capability of *de novo* synthesizing Fatty Acids (FA), myocytes rely on the supply of FA from extracellular sources to cover their need of these substrates. Fatty acids are supplied to cardiac and skeletal muscle either bound to plasma albumin (non-esterified fatty acids) or in the form of triacylglycerols in the core of circulating lipoproteins (Van der Vusse *et al*., 1992). Moreover, changes in the muscle content of FA most likely indicate profound changes in muscle metabolism in general and in FA handling in particular. Since, the metabolic fate of FA in cardiac and skeletal muscle may depend on chain length and degree of unsaturation (Van der Vusse *et al*., 1992; Van der Vusse and Reneman, 1996), it is of importance to analyze the relative composition of the FA pool in these tissues. Fatty acids not only substantially contribute to muscle oxidative energy conversion, but also serve as substrates for biological active compounds, such as eicosanoids and membrane phospholipid synthesis and act as ligands for protein factors involved in signalling transduction and gene expression (Pompeia *et al*., 2003).

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Insulin's effects are associated with energy metabolism, largely involving the regulation of glucose and fatty acid metabolism. Indeed, insulin plays a vital role in the regulation of glucose and fatty acid homeostasis and an important role in amino acid metabolism. As such, it facilitates the storage of fuels and macromolecules in liver, muscle and adipose tissues (Steinberg and Baron, 2002). In diabetic patients, since Δ^6 desaturation pathway is inadequate, essential and non essential fatty acid metabolism is acquired (Horrobin, 1993).

Mimouni and Poison (1992) reported that desaturase activities, which are partially inhibited by spontaneous diabetes during the normo-and hyper-glycemic periods, were similarly affected by the various insulin treatment; Δ^9 desaturase activity being more depressed than the desaturase activities of either Δ^6 of Δ^5. Insulin treatment with 10 IU kg^{-1} body weight twice a day for 2 days was able to restore the $\Delta^{9,6,5}$ desaturase activities to control levels during the hypoglycemia period.

It is reported that diabetes induced a decrease of monounsaturated fatty acids and particularly palmitoleic acid in plasma liver and aorta (Douillet and Ciavatti, 1995). Shin *et al.* (1995) show that the fatty acid composition of the liver and the erythrocytes and examined Δ^6 desaturase activities to compare the effect of short-term insulin therapy on the tissues with and without Δ^6 desaturase, i.e., the liver and the erythrocytes using STZ induced diabetic rats.

Brenner *et al.* (2000) reported that streptozotocin diabetes depresses $\Delta^{9,6,5}$ fatty acid desaturases, decreasing arachidonic acid and increasing linoleic acid, but also unexpectedly increasing docosahexaenoic acid in the different phospholipids of liver microsomal lipids. Brenner (2003) showed that in experimental diabetes mellitus type-1, the depressed Δ^6 desaturase is restored by insulin stimulation of the gene expression of its mRNA. Rimoldi *et al.* (2001) reported that streptozotocin-induced diabetic rats in order to determine the regulatory role of insulin on the expression of hepatic Δ^6 desaturase gene. The abundance of hepatic Δ^6 desaturase mRNA in the diabetic rats is seven-folds lower than in the control.

In this research, it was aimed to examine that the effects of VC, LA and their comibination on the fatty acid composition of lung and muscle tissues of poor controlled diabetic Wistar rats.

MATERIALS AND METHODS

Animal selection treatment: A total of 46 male Wistar rats (200-250 g), supplied by Elazig Animal Diseases Research Central of the Agricultural Ministry of Turkey and were used in this study. Rats were housed in cages, where they had *ad libitum* rat chow and water in an air-condition room with 12 h light/12 h dark cycle. Animals were randomly divided into five groups. The first group was used as a control (n = 6), the second group diabetes (n = 10), the third group Vitamin C + D (n = 10), the fourth group LA + D (n = 10), the fifth group, LAVC + D (n = 10). Rats in D, VC + D, LA + D and LAVC + D groups were made diabetic a single intraperitoneal injection of 45 mg kg^{-1} Streptozotocin (STZ) in citrate buffer (pH = 4.5). Control group rats were injected intraperitoneal with buffer alone. Later 2 days after administration of STZ, tail vein blood glucose level was measured in all the animals. Blood glucose level was 250 mg dL^{-1} and above was considered diabetics. After diabetic formation, no insulin was injected to rats. The rats in D + VC group were injected intraperitoneal (ip) 100 mg kg^{-1} L-ascorbic Acid (VC) 4 times $week^{-1}$. The rats in LA + D group were received 50 mg kg^{-1} lipoic acid, the rats in LAVC + D group was injected 100 mg kg^{-1} VC and 50 mg kg^{-1} LA via ip. These treatments were continued for 6 weeks, after which time each experimental rat was anesthetized with ether and liver and lung tissue samples were collected and stored in -85°C prior to biochemical analyses.

Extraction of lipids and preperation of fatty acid methyl esters: The lipids of muscle and lung tissue samples were extracted by the method of Hara and Radin (1978). Tissue samples were homogenized with the mixture of hexane: isopropanol (3:2, v v^{-1}) in the homogenizator. Fatty acids of the lipid extract were converted to methyl esters by using 2% sulfuric acid (v v^{-1}) in methanol (Christie, 1992).

Fatty acid methyl ester forms were extracted with n-hexane. Analysis was performed in a Shimadzu GC-17A V3 intstrument gas chromatograph equipped with a Flame Ionization Detector (FID) and a 25 m, 0.25 mm i.d. permabond fused-silica capillary column (Machhery-Nagel, Germany). The oven temperature was programmed between 120-220°C, 5°C min^{-1}. Injector and FID temperatures were 240 and 280°C, respectively. The nitrogen carrier gas flow was 1 mL min^{-1}. The methyl esters of fatty acids were identified by comparison with authentic external standard mixtures analyzed under the same conditions. Class GC 10 software version 2.01 was used to process the data. The results were expressed as percent.

Statistical analysis: The experimental results were reported as means±SD. Statistical analysis was performed using SPSS software. Analysis of Variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls.

RESULTS

Acids including myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, eicosacoic acid, eicosatrienoic acid, arachidonic acid,

eicosapentaenoic acid, docosatetraenoic acid, docosapentaenoic acid and docosahexaenoic acid were observed in fatty acid components of the lung. Of these palmitic, stearic, oleic, linoleic and arachidonic acids were present in higher quantities in animals of the control group (Table 1).

The comparative transition analysis of fatty acids show that the quantity palmitic acid was higher in the LA + D group ($p<0.05$) but that palmitoleic acid was higher within the diabetes and other groups compared with the control group ($p<0.01$). Although, the quantity of stearic acid was higher in diabetes and other groups compared with the control group ($p<0.001$), the quantity of oleic acid decreased ($p<0.0001$) (Table 1).

Although, the quantity of linoleic decreased in diabetes and other groups compared with the control group ($p<0.001$), eicosatrienoic asit, arachidonic acid, docosapentaenoic acid and docosahecsaenoic acid levels increased compared with the control group ($p<0.01$, $p<0.001$) (Table 1).

Fatty acid of muscle tissue was composed of palmitic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid, docosapentaenoic acid and docosahexaenoic. The statistical transition analysis of these acids showed that the quantity of palmitic acid decreased in diabetes and other groups compared with the control group ($p<0.05$). Although, the quantity of stearic acid increased in diabetes and other groups compared with control group ($p<0.01$), oleic acid (18:1, n-9) and palmitoleic (16:1, n-7) decreased ($p<0.01$, $p<0.001$). Although, linoleic acid was higher in diabetes and VC + D groups compared with the control group ($p<0.01$), there was slighly difference in the LA + D and LAVC + D groups ($p<0.05$) (Table 2).

The quantity of arachidonic acid was the same in the diabetes, VC + D and control groups but was higher in the LA + D and LAVC + D groups ($p<0.01$). Both docosapentaenoic acid (22:5, n-3) and docosahexaenoic acid (22:6, n-3) were higher in the diabetes and other supplemented groups compared with the control group (Table 2).

Table 1: Fatty acid composition of lung tissue (%)

Fatty acids	Control	Diabetes	VC + D	LA + D	VCLA + D
14:00	1.81±0.07	1.07±0.05	1.15±0.09	1.48±0.15	1.27±0.12
16:00	30.74±0.82	29.72±0.81	30.50±1.06	34.23±1.97[a]	32.90±1.55
18:00	9.09±0.44	12.81±0.30[c]	12.46±1.39[c]	11.59±0.61[c]	11.99±0.47[c]
16:1, n-7	0.90±0.07	1.13±0.03[b]	1.23±0.09[b]	1.51±0.14[b]	1.43±0.10[b]
18:1, n-9	22.42±1.53	12.30±0.63[d]	13.67±0.73[d]	11.72±0.75[d]	12.58±0.64[d]
18:1, n-7	2.55±0.04	2.40±0.11	2.22±0.13	2.11±0.14	2.11±0.09
18:2, n-6	17.67±1.03	14.29±0.93[b]	15.85±0.64[b]	13.11±0.69[b]	14.71±0.56[b]
20:2, n6	0.33±0.02	0.54±0.04	0.63±0.02	0.50±0.02	0.71±0.04
20:3, n-6	0.37±0.03	0.84±0.03[d]	0.80±0.04[d]	0.82±0.04[d]	0.91±0.05[d]
20:4, n-6	8.67±0.96	16.95±1.11[d]	10.66±0.36[b]	11.88±0.90[b]	11.14±0.52[b]
20:5, n-3	0.20±0.01	0.70±0.07[d]	0.69±0.04[d]	0.82±0.06[d]	0.86±0.03[d]
22:4, n 6	2.10±0.24	1.92±0.11	1.60±0.06	1.81±0.19	1.93±0.13
22:5, n 3	1.34±0.04	2.01±0.10[c]	2.62±0.05[c]	3.23±0.13[d]	2.21±0.09[c]
22:6, n3	2.66±0.04	5.89±0.26[d]	6.11±0.27[d]	5.39±0.33[d]	5.19±0.14[d]
Σ saturated	41.64	41.60	44.91	47.30	46.16
Σ unsaturated	59.36	58.40	56.38	52.70	53.84
ΣPUFA	34.34	41.14	38.86	37.56	37.66
ΣMUFA	25.45	15.83	17.12	14.34	16.12
Σ ω 3	04.20	08.60	09.12	09.44	08.26
Σ ω 6	30.04	32.54	29.44	28.12	29.40

Table 2: Fatty acid composition of muscle tissue (%)

Fatty acids	Control	Diabetes	VC + D	LA + D	VCLA + D
16:00	23.46±0.21[a]	20.67±0.43[b]	20.58±1.14[b]	19.42±0.31[b]	21.93±1.68[a]
18:00	9.44±0.56	12.53±1.06[c]	12.10±1.22[c]	15.17±0.58[d]	14.47±0.67[d]
16:1, n-7	3.64±0.16	0.83±0.07[d]	0.60±0.11[d]	0.40±0.12[d]	0.50±0.14[d]
18:1, n-9	22.20±1.38	14.66±1.99[c]	15.08±2.43[c]	7.65±0.94[d]	9.31±1.61[d]
18:1, n-7	2.88±0.09	2.71±0.09	2.66±0.19	2.22±0.12	2.17±0.14
18:2, n-6	24.29±0.57	28.30±1.11[c]	29.13±0.84[c]	26.73±0.71[b]	26.29±2.11[b]
20:4, n-6	7.55±1.12[a]	7.00±1.08[a]	8.28±1.11[a]	12.26±0.90[d]	11.55±1.23[d]
22:5, n-3	0.77±0.09	1.47±0.19[b]	1.28±0.14[b]	2.04±0.12b	1.84±0.16[b]
22:6, n-3	5.67±0.61	13.01±1.22[d]	10.39±1.26[c]	14.70±0.55[d]	12.45±1.10[d]
Σ saturated	33.29	33.00	32.48	34.59	37.40
Σ unsaturated	66.71	67.38	67.32	66.00	63.60
ΣPUFA	38.30	48.78	49.02	55.73	52.13
ΣMUFA	28.72	18.20	18.26	10.27	11.48
Σ ω 3	6.44	13.48	11.67	16.74	14.29
Σ ω 6	31.86	35.30	37.41	38.99	37.84

[a]$p>0.05$; [b]$p<0.05$; [c]$p<0.01$; [d]$p<0.001$

DISCUSSION

Type 1 diabetes mellitus is characterized by loss or deficiency of insulin. Loss of insulin does not affect all metabolisms of tissues in the same way and some tissues can be affected over the long term (Huang *et al*., 1984; Poisson *et al*., 1993; Shin *et al*., 1995; Alberti and Zimmet, 1998). Muscle tissue is particularly sensitive to loss of insulin, as there are no enzyme units capable of synthesizing fatty acids in muscle tissues. Muscle tissues obtain essential and nonessential fatty acids from the bloodstream. Loss of insulin prevents the functioning of proteins responsible for glucose uptake by muscle tissues and also affects the proteins responsible for transference of fatty acids (Taylor and Agius, 1988). In the present study, a diabetes model was designed but insulin was not used daily. The efficiency of vitamin C and α-lipoic acid on products of desaturase enzymes of diabetic rats were analyzed.

The results show that the quantity of palmitic acid in lung tissue fatty acid composition partially increased in LA + D and LA + VC + D groups compared with the control group (Table 1). This increase was due to the effect of lipoic acid. The effect of lipoic acid results from metabolic features rather than antioxidant features. As lipoic acid is the cofactor of pyruvate dehydrogenase enzyme of the energy metabolism, it increases the turnover of the Tricarboxylic Acid (TCA) cycle and indirectly triggers the formation of citrate molecules which are used in the synthesis of fatty acids (Biewenga *et al*., 1997; Bisby and Parker, 1998; Suzuki *et al*., 1991). The increase of palmitic acid in lung tissue, which is less sensitive to insulin in glucose uptake from blood than muscle tissue, can be a result of this effect. This effect is not observed in muscle tissue because other factors than insulin are not active in glucose uptake from the blood.

The quantity of palmitoleic acid in lung tissue partially increased in the diabetes and other groups compared with the control group. However, the analysis of composition of fatty acids in the same tissue showed that the quantity of oleic acid decreased noticeably in other groups compared with the control group. The decrease of these fatty acids results from a deficiency in the activation Stearoyl CoA Desaturase (SCD) enzyme. Stearoyl CoA enzyme forms palmitoleic acid (16:1, n-7) and oleic acid (18:1, n-9) by using palmitic acid (16:0) and stearic acid (18:0) as a substrate (Douillet and Ciavatti, 1995; Nitambi and Miyazaki, 2004; Cheul and Nitambi, 1999).

The results of oleic acid in both lung and muscle tissue that antioxidant agents were ineffective. The application of lipoic acid in muscle tissue decreased the quantity of palmitoleic and oleic acid. The quantity of stearic acid was high in both tissues. These results from pressure applied on SCD enzymes due to loss of insulin (Table 1 and 2). The analysis of lipid metabolism and fatty acid metabolism showed that the quantity of palmitic, stearic and linoleic acid was higher but the quantity of synthesized fatty acid was lower in tissues. This is the apparent feature of insulin deficiency, triggered by diabetes. As insulin was not used in the present study, the synthesis of SCD enzyme decreased and phase of these enzymes was not fully provided. The results of the present study are similar to previous studies in the literature (Brenner, 2003; Montanaro *et al*., 2003; Attie *et al*., 2002; Cheul and Nitambi, 1999; Douillet and Ciavatti, 1995). Chorvathova and Ondreicka (1983) assessed that palmitoleic acid decreased in adipose tissue and serous fluid and oleic acid decreased in the renal cortex, lung and muscle tissue.

Fatty acid metabolism composed of synthesized fatty acid and SCD enzyme is also known as endogenous fatty acid and essential fatty acid. Fatty acid metabolism composed of linoleic (18:2, n-6) and linolenic (18:3, n-3) acids are known as essential fatty acids. Essential fatty acid is composed of Δ^6 and Δ^5 desaturase enzyme and Δ^6 desaturation is added to this acid. The results of activation of these enzymes are γ-linoleic, eicosatrienoic, arachidonic, docosapentaenoic and docosahexaenoic acids (Horrobin, 1989).

The results of the present study showed that while linoleic acid decreased, arachidonic acid increased in lung tissue. Linoleic acid increased in all groups compared with the control group but arachidonic acid increased only in the muscle tissue samples of the D + LA and D + LA + VC groups. Docosapentaenoic and docosahexaenoic acids were higher in both lung and muscle tissue when compared with the control group.

High polyunsaturated fatty acids such as arachidonic acid and docosahexaenoic acid are essential for brain development, heart functions, inflammatory responses and balance. Δ^6 and Δ^5 desaturase enzymes are a rate limiting step in the synthesis of arachidonic acid and docosahexaenoic acid (Soldati *et al*., 2002; Youdim *et al*., 2000). Diet, hormonal balance, various illnesses and genetic factors are effective on the Δ^5 desaturase enzyme (Zolfaghari and Ross, 2003). The excessive increase of arachidonic acid causes Ca^{++} ions, which have a cytotoxic effect in cells (Pompeia *et al*., 2003).

Animal biosynthesis of high polyunsaturated fatty acids from linoleic, α-linolenic and oleic acids is mainly modulated by the Δ^6, Δ^5 and Δ^9 desaturases through dietary and hormonally stimulated mechanisms. For hormones, only insulin activates both enzymes. In experimental diabetes mellitus type 1, the depressed Δ^6 desaturase is restored by insulin stimulation of the gene expression of its mRNA (Brenner, 2003).

The depression of Δ^6 and Δ^5 desaturases in diabetes is rapidly correlated by lower contents of arachidonic acid and higher contents of linoleic acid in almost all tissues except the brain. However, docosahexaenoic acid enhancement, mainly in liver phospholipids, is not yet fully understood (Gurr and Harwood, 1991).

Docosahexaenoic acid (22:6) is a polyunsaturated fatty acid that is abundant in all tissues. The fatty acid has various functions, such as brain development, transference of nerve impulses and eyesight (Youdim *et al*., 2000; Horrocks and Yeo, 1999). Docosahexaenoic acid decreases in case of alcohol syndrome, hyperactive disorders, cystic fibrosis, phenylketonuria and metabolic disorders (Horrocks and Yeo, 1999). Both arachidonic acid and docosahexaenoic acid have important functions in all tissues. However, the excessive increase of arachidonic acid is characterized as an abnormal metabolic case (Pompeia *et al*., 2003).

Consequently, the molecular analysis of antioxidant supplementation showed that their effect is minimal, or they do not have any effect in an insulin-dependent diabetes model. The antioxidant application is completely inefficient in tissues requiring high levels of insulin.

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