Consequences of the Combined α-tocopherol, Ascorbic Acid and α-lipoic Acid on the Glutathione, Cholesterol and Fatty Acid Composition in Muscle and Liver of Diabetic Rats

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ABSTRACT

Objective(s): Our objective was to evaluate the effects of a triple antioxidant combination [α-tocopherol (AT), ascorbic acid (AA) and α-lipoic acid (LA); AT+AA+LA] on the cholesterol and glutathione levels, and the fatty acid composition of liver and muscle tissues in diabetic rats.

Materials and Methods: Forty-three Wistar rats were randomly divided into five groups. The first group was used as a control. The second, third and fourth groups received STZ (45 mg/kg) in citrate buffer. The fourth and fifth groups were injected with intraperitoneal (IP) 50 mg/kg DL-AT and 50 mg /kg DL-LA four times per week and received water-soluble vitamin C (50 mg/kg) in their drinking water for a period of six weeks.

Results: Liver cholesterol levels in the AT+AA+LA group were lower than the control ($P<0.05$). Glutathione level was lower in D-2 ($P<0.05$) and were higher in D+AT+AA+LA and AT+AA+LA groups than the control groups ($P≤ 0.05$). The muscle cholesterol levels in the D-1 and D+AT+AA+LA groups were higher than the control group ($P≤ 0.05$). The levels of oleic acid were higher in the D-1 group and lower in the D-2 group ($P<0.001$). The arachidonic acid level in the D-1 and D-2 groups were lower ($P<0.05$), and higher in the D+AT+AA+LA group.

Conclusion: Our results revealed that glutathione levels and the Stearoyl CoA Desaturase enzyme products in liver tissues of diabetic and non-diabetic rats were increased by triple antioxidant mixture.

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Introduction

Diabetes mellitus is a common degenerative disease as one of the leading global causes of morbidity and mortality (1). Increased oxidative stress and impaired antioxidant defense mechanisms are important factors in the pathogenesis and progression of diabetes (2). Clinically, diabetes is a heterogeneous disease, with a common phenotype of impaired glucose tolerance; depending on the basis of the management required to control glucose homeostasis, it can be divided into type 1 and type 2 diabetes (1, 3, 4). In the long term, diabetes leads to complications in numerous organs (5).

Evidence from experimental animals has shown the effect of diabetes on fatty acid composition liver and muscle tissues (6). Since muscle tissue lacks the capability of *de novo* synthesis of fatty acid (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 (7). Furthermore, 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 (8), it is important to analyze the relative composition of the FA pool in these tissues. FAs are not only substantially contribute to muscle oxidative energy conversion, but also serve as substrates for biologically active compounds, such as eicosanoids, and membrane phospholipid synthesis, and act as ligands for protein factors involved in signaling transduction and gene expression. Borkman *et al* (9) stated that skeletal muscle is a main site of insulin action, and insulin sensitivity may be related to the fatty-acid composition of the phospholipids within the muscle membranes. Declined insulin sensitivity is associated with decreased concentrations of polyunsaturated fatty acids in skeletal-muscle phospholipids, raising the possibility that changes in the fatty-acid composition of muscles modulates the action of insulin. Lipid metabolism in the liver is very complex procedure and involves, among many other functions, the synthesis and secretion of very low density lipoproteins, ketone bodies and a high rate of fatty acid oxidation, which provides most of the energy that the organ requires in order accomplishing its many functions in the organism (10). Poisson and Cunnane (11) suggested that metabolism of long-chain fatty acids is impaired in both fasting and diabetes. The decrease in insulin activity simultaneously reduces the activity of the Δ-9, Δ-6, and Δ-5 desaturases with respect to fatty acids either synthesized by the animal (palmitic and stearic acids) or dietary essential (linoleic and alpha-linolenic) acids. Mimouni and Poisson (12) reported that Δ-9 desaturase activity was more depressed than the desaturase activities of either Δ-6 of Δ-5, and that insulin treatment with 10 IU/kg body weight twice a day for 2 days was able to restore the Δ- 9, 6 and 5 desaturase activities to control levels during the hypoglycemicstage . Douillet and Ciavatti (13) found that diabetes induced a decrease of monounsaturated fatty acids and particularly palmitoleic acid (16:1 n-9) in liver, aorta and plasma. They suggested that AT partially corrected Δ-9 desaturase activity and consequently enhanced monounsaturated acids in all the tissues. In addition, vitamin E treatment was normalized Δ-6 desaturase activity in the liver, increased eicosatrienoic acid (20:3 n-6) and arachidonic acid (20:4 n-6, AA) levels in the aorta. Shin et al (6) revealed that the effect of insulin on the fatty acid composition of the liver and the erythrocytes in using STZ-induced diabetic rats. They found that the activities of Δ-6 desaturase in diabetic rats

were 68% of those of controls and increased to 119% of controls following insulin treatment. It has been suggested that insulin restores the fatty acid composition earlier in the liver microsome in STZ-induced diabetic rats.

Lipoic acid plays an important role in energy metabolism. It is involved in different multienzyme complexes such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched-chain α-keto acid dehydrogenase, and glycine decarboxylase complex (14, 15). Jacop *et al* (16) have indicated that parenteral administration of the antioxidant α-LA significantly enhances the capacity of the insulin-stimulated glucose transport system and of both oxidative and nonoxidative pathways of glucose metabolism in the skeletal muscle of insulin-resistant rats. Alpha tocopherol is the major chain-breaking antioxidant that protects membranes from lipid peroxidation. However, AA and reduced glutathione (GSH) will act as tocoperoxyl radical scavengers only when there is efficient removal of their oxidised forms. GSH and ascorbat circumvent lipid peroxidation via the reduction of tocopheroxyl radical to tocopherol (17). It has been reported that antioxidants such as glutathione, AT, AA, and antioxidant enzymes superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidases exert synergistic actions in scavenging free radicals (18). Evidence from *in vitro* studies has suggested that antioxidant vitamins can prevent lipid peroxidation and that AA and AT have synergistic effects. However, *in vivo* evidence in support of these hypotheses is sparse (19).

Much evidence has indicated oxidative stress as a major source of insulin resistance, whereas contemporary dietary recommendations often lack reliable information indicating the benefits of a considerable increase of antioxidants (AT, AA, LA) in the diet of subjects belonging to the high-risk group for the development of insulin resistance (20). The aim of this study was to examine the effects of triple combination of AT+AA+LA on the total cholesterol, reduced GSH levels, and fatty acid composition (products of SCD and Δ^6 and Δ^5 desaturase enzymes) in the liver and muscle tissues of diabetic rats.

Materials and Methods

Animals, diets, experimental design

A total 43 Wistar rats (200–250 g; 10 weaks old) were used. The animals were housed in cages in an air-conditioned room with a 12 hour light/dark cycle where they had *ad libitum* access to rat chow and water. Animals were randomly divided into five groups. The first group was used as a control (n = 6), the second group D-1 (n = 10), the third group D-2 (n = 10), the fourth group D+AT+AA+LA (n = 10), the fifth group AT+AA+LA (n =7). Rats in D-1, D-2, and D+AT+AA+LA groups were made diabetic via a single intraperitoneal injection (IP) of 45 mg/kg STZ in citrate

buffer (pH = 4.5). Control group were injected IP with the buffer alone. Two days following administration of STZ, the tail vein blood glucose level was measured in all the animals. Those with blood glucose level of 250 mg/dl and above were considered diabetics. Following formation of diabetic, rats in D-1 and D+AT+AA+LA groups received daily subcutaneous injections of 8 IU/kg insulin; rats in the D-2 group were not injected. Rats in the D+AT+AA+LA group were injected intraperitoneal (IP) 50 mg / kg DL-AT and 50 mg/kg DL-LA four times per week and received water-soluble vitamin C (50 mg/kg) in their drinking water. These treatments were continued for six weeks, after which each rat was anesthetized with ether, liver and muscle tissue samples were collected and stored at -85°C prior to biochemical analyses.

Laboratory analyses

Lipids were extracted with chloroform-methanol (2:1, v/v) using the method of Folch *et al* (21) as described previously (22). Liver and muscle tissues were homogenized with the mixture of chloroform/methanol in homogenizator. Aliquots were taken and the total cholesterol quantified by high performance liquid chromatography (23). Aliquots of each extract were saponified and the fatty acids esterified with 2% H_2SO_4 in methanol (22) and the fatty acid composition determined by gas chromatography.

Cholesterol analysis

For the cholesterol analysis, fully automatic HPLC (Shimadzu, Kyoto Japan) had been used. A Supelcosil LC 18TMDB column (250 x 4.6 mm, 5 μm, Sigma USA) was used as the HPLC column. The mobile phase used was acetonitrile – isopropyl alcohol (70:30 v/v) at a flow rate of 1 ml/ min (23). Detection was performed by UV at 202 nm and 40°C column oven (24). For sample preparation, one ml of the hexane extract obtained by direct saponification was dried under nitrogen flow and diluted with a 500 μl of the mobile phase. The mixture was injected via auto sampler to the HPLC instrument. Quantification was carried out by external standardization using *Class VP software*. The results were expressed as μmol/g tissue weight.

Determination of GSH in Liver and muscle tissues

For the GSH analysis, a Discovery RP-Amide C16 column (150 x 4.6 mm, 5 μm) was used and 50 mM NaClO4 0.1 % H_3PO_4 was used as the mobile phase with a flow rate of 1 ml/min (25). Detection was performed at 215 nm by UV-Visible detector and 40 C column oven. 0.2 g liver and muscle tissue samples were homogenized with the mixture of 2 ml 10 mM ETDA and 50 mM NaClO4. Following deproteinization with 5 % metaphosphoric acid (26), samples were centrifuged at 4°C and 12,000 x g for 10 min. The supernatants were injected via an autosampler

to the HPLC. Quantification was carried out by external standardization using *Class VP software*. The results were expressed as μmol/g tissue weight

Fatty acid methyl ester analysis

Fatty acids in lipid extracts were converted to methyl esters by using 2% sulfuric acid (v/v) in methanol (22). Fatty acid methyl ester forms were extracted with n-hexane. Gas Chromatography (GC) analysis was employed from Shimadzu (Kyota, Japan). Methyl ester mixtures were separated by a capiller column, Permabond (Machery-Nagel, Germany). Column temperature was programmed between 130 – 220°C, 5°C/min and the final temperature was held for 15 min. Injector and FID temperatures were 240 and 280°C, respectively. Individual methyl esters 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 tissue weight.

Statistical analysis

The experimental results were reported as mean ± SE. Statistical analysis was performed using SPSS. Analysis of variance (ANOVA) and Least Significant Difference (LSD) test were used to compare the experimental groups with the controls.

Results

Total lipid, cholesterol and reduced glutathione (GSH) levels

The effects of triple antioxidant combination on the cholesterol and GSH levels of liver are shown in Table 1. Cholesterol level did not differ between the D+AT+AA+LA and control groups (P>0.05), however, the cholesterol levels in D-1, D-2, and AT+AA+LA were lower than the control group (respectively, P< 0.05, P< 0.01). While the GSH level did not differ between the control and D-1 groups, its level decreased in the D-2 group (P<0.05). However, the GSH level increased in D+AT+AA+LA and AT+AA+LA groups (P<0.05, P<0.01).

Muscle cholesterol level was increased in D-1,

Table 1. The levels of total lipid, cholesterol and GSH in liver tissue

Groups	Total cholesterol μmol / g tissue	Reduced glutathione (GSH) μmol / g tissue
Control	9.00 ±0.49 [a]	0.95 ± 0.12 [a]
D-1	8.35 ± 0.56 [a]	1.06 ± 0.06 [a]
D-2	5.77 ±0.50 [b]	0.65 ± 0.05 [b]
D+AT+AA+LA	8.69 ±0.56 [a]	1.43 ± 0.28 [b]
AT+AA+LA	5.59 ±0.51 [c]	1.86 ± 0.09 [c]

D+AT+AA+LA and AT+AA+LA groups ($P<0.05$). However, the cholesterol level decreased in the D-2 group ($P<0.01$). Although GSH level did not differ between the control, D-1 and D+AT+AA+LA groups, its level decreased in the D-2 group, but its level in the AT+AA+LA group was higher than the other groups (Table 2). Fatty acid composition
As revealed in Table-3, palmitic acid level (16:0) in the

fatty acid composition of the liver increased in D-2, D+AT+AA+LA and AT+AA+LA groups compared to control ($P<0.001$), but stearic acid level decreased in the same groups ($P<0.001$). While the liver palmitoleic acid level (16:1, n-9) did not differ between control, D-1 and D-2 groups, its level increased in the D+AT+AA+LA group ($P<0.01$). Although liver oleic acid level was high (18:1, n-9) in the D+AT+AA+LA and AT+AA+LA groups ($P<0.01$), its level decreased in the D-2 group ($P<0.05$). Liver linoleic acid level (18:2, n-6 LA) was high in the D-1, D- 2 and D+ AT+AA+LA groups ($P<0.01$), however arachidonic acid (20:4, n-6) level was low in the D-2 and D+ D+ AT+AA+LA groups ($P<0.001$). Docosahexaenoic acid (22:6, n-3, DHA) level increased in the D-2 and AT+AA+LA groups (respectively, $P<0.001$, $P<0.05$), although there was no differences between control, D-1 and D+AT+AA+LA groups ($P>0.05$) (Table 3).

Table 4 presents changes in muscle fatty acid composition by triple antioxidant combination. The muscle stearic acid level was high in the D-2, D+AT+AA+LA and AT+AA+LA groups ($P<0.01$, $P<0.001$). Muscle oleic acid levels increased in the D-1 group, but its level decreased in the D-2 group ($P<0.001$). While the linoleic acid levels of muscle was high in the D-1 and D-2 groups ($P<0.01$),

Table 2. The levels of total lipid, cholesterol and GSH in muscle tissue

Groups	Total cholesterol μmol / g tissue	Reduced glutathione (GSH) μmol / g tissue
Control	4.29 ± 0.80 [a]	0.93 ± 0.14 [a]
D-1	6.80 ± 0.75 [b]	0.89 ± 0.16 [a]
D-2	2.82 ± 0.45 [c]	0.59 ± 0.11 [b]
D+AT+AA+LA	7.35 ± 0.90 [b]	0.97 ± 0.32 [a]
AT+AA+LA	6.72 ± 0.75 [b]	1.24 ± 0.18 [b]

Values are the mean ± SE. ANOVA; one-way ANOVA P value:
a: $P>0.05$, b: $P<0.05$, c: $P<0.01$, d: $P<0.001$ using the post hoc Fischer's P LSD test.
D-1: During the of diabetes was injected the insulin (8 IU/kg)
D-2: During the of diabetes was not injected insulin
AT+AA+LA = combination of a-TOC, ASA and a-LA
D+AT+AA+LA= Diabetes+ combination of a-TOC, ASA and a-LA

Table 3. The fatty acid composition in liver tissues of diabetic rats

Fatty acids	Control	D-1	D-2	D+ AT+AA+LA	AT+AA+LA
14:0	0.81±0.05	0.24 ±0.05	0.18±0.05	0.48±0.05	0.83±0.21
16:0	16.89±0.30	20.95±1.07[d]	22.22±0.99 [d]	22.23±1.05[d]	18.08±0.77[b]
18:0	24.45±1.23	18.38±0.88[d]	19.75±1.08 [d]	16.45±0.19[d]	21.19±0.78[b]
S Saturated	41.55±0.93	39.57±1.00[b]	38.00±1.30[b]	39.16±1.20[b]	40.10±1.65[a]
16:1, n 9	1.04±0.20[a]	0.98±0.35[a]	0.97±0.43[a]	2.50±0.59[d]	1.53±0.33 [b]
18:1, n 9	10.08±1.17[a]	10.22±0.14[a]	7.11±1.40[b]	15.45±1.24[c]	9.76±0.51[d]
18:1, n 7	2.11±0.35[a]	2.62±0.15[a]	3.16±0.39[b]	2.65±0.39[b]	2.69±0.12[a]
S MUFA	13.23±1.23[a]	13.82±0.92[a]	11.24±1.16[a]	20.60±0.82[d]	13.98±1.20[a]
18:2	14.39±0.80	17.32±0.47 [c]	19.74±1.79 [d]	16.37±0.75 [b]	18.01±0.42[c]
20:4	23.50±0.70 [a]	22.82±2.33 [a]	14.31±1.03 [d]	17.97±0.18 [c]	19.81±0.54[c]
22:6	6.76±0.64 [a]	6.47±0.36 [a]	12.74±1.53 [b]	5.90±0.42 [a]	8.12±0.11[a]
S PUFA	44.65±2.22	46.61±3.33 [b]	46.79±2.51[b]	40.24±1.50[b]	45.88±1.40[a]
S Unsaturated	58.11±1.22	60.43±2.12 [b]	58.03 ±1.82 [a]	60.84±2.00 [b]	60.02±2.21[a]
18:1/18:0	0.50	0.70	0.61	1.10	0.54
16:1/16:0	0.062	0.047	0.046	0.112	0.080
S Unsa /S Sat	1.40	1.53	1.63	1.55	1.44

Values are the mean ± SEM. ANOVA; one-way ANOVA P value:
a: $P>0.05$, b: $P<0.05$, c: $P<0.01$, d: $P<0.001$ using the post hoc Fischer's P LSD test
S Saturated: Total saturated fatty acids
S Unsaturated: Total unsaturated fatty acids
S MUFA: Total monounsaturated fatty acids
S PUFA: Total polyunsaturated fatty acids

Table 4. The fatty acid composition in muscle tissues of diabetic rats

Fatty acids	Control	D-1	D-2	D+ AT+AA+LA	AT+AA+LA
14:0	0.96±0.15	0.87±0.22	0.86±0.33	0.79±0.20	0.87±0.18
15:0	0.74±0.21	0.34±0.14	0.40±0.12	0.45±0.10	0.90±0.15
16:0	23.46±1.22[a]	22.01±0.26[a]	22.48±0.43[a]	22.57±0.69[a]	22.95±0.58[a]
18:0	9.49±0.91[a]	9.33±0.12[a]	13.43±1.06[d]	10.89±0.52[b]	10.88±1.12[c]
S Saturated	34.65±1.13[a]	32.55±1.18[b]	37.17±0.89[b]	34.70±1.46[a]	36.67±1.93[b]
18:1, n 9	22.40±0.99[a]	24.12±092[c]	15.57±1.99[d]	21.73±1.40 [a]	20.62±1.49[b]
18:1, n 7	6.08 ± 0.74	3.71±0.24[d]	3.61±0.19 [d]	4.31±0.42[c]	4.38±0.29 [b]
S MUFA	28.77±1.80[a]	28.97±1.20[a]	19.18±2.00 [d]	26.04±1.75 [b]	25.00±1.60[b]
18:2	21.50±0.74[a]	27.85±0.93c	28.30±1.11 [c]	20.42±1.59[a]	20.24±0.77[a]
20:4	8.54±026[a]	7.08±065[b]	7.00±1.09 [b]	10.20±0.88 [b]	9.39±043[a]
22:6	6.67±0.50	4.77±0.83[b]	8.41±1.22 [b]	8.73±0.49 [b]	9.86±0.93[c]
S PUFA	36.40±1.50	39.63±2.00[b]	43.31±2.08 [d]	39.35±2.48 [b]	39.49±1.50 [b]
SUnsaturated	65.38±2.20[a]	67.46±1.86[b]	62.89±1.60 [b]	65.39±1.90[a]	64.49±1.75[b]
18:1/18:0	2.95	2.98	1.42	2.39	1.70
S Unsa /S Sat	1.89	2.07	1.66	1.88	1.59

Values are the mean ± SEM. ANOVA; one-way ANOVA P value. a $P>0.05$, b: $P<0.05$, c: $P<0.01$, d: $P<0.001$ using the post hoc Fischer's P LSD test.
S Saturated: Total saturated fatty acid
S Unsaturated: Total unsaturated fatty acid
S MUFA: Total monounsaturated fatty acid
S PUFA: Total polyunsaturated fatty acid

muscle arachidonic acid level was low in the same groups ($P<0.05$). However, muscle arachidonic acid level increased in the D+AT+AA+LA and AT+AA+LA groups ($P<0.05$). While DHA level in muscle decreased in the D-1 group, its level increased in the D-2 and AT+AA+LA groups ($P<0.05$) (Table 4).

Discussion

Experimental and clinical studies revealed that free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins (2, 27). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage to cellular organelles and enzymes, increased lipid peroxidation (2), and abnormal lipid, triglycerides and cholesterol levels (28). The polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are subject to free radical-initiated oxidation and contribute in chain reactions that amplify damage to biomolecules. The present study indicated that cholesterol level in muscle was high in the insulin treated D-1 group. Muscle is a major site of insulin action, and insulin sensitivity may be related to the cholesterol level of muscle lipids. Insulin increases activity of the 3-hydroxy-3-methylglutaryl coenzyme A *reductase* enzyme (HMG-CoA), which is a key enzyme in cholesterol biosynthesis.

Therefore, cholesterol synthesis increases and facilitates the storage of fuels and macromolecules in liver, muscle and fat (29). In the present results, the cholesterol level was also found to be high in the insulin and triple antioxidant treated diabetic groups. This augmentation might be a result of insulin administration. However, the level of cholesterol in the liver tissues of AT+AA+LA group was lower than the control group. This decrease may be due to the effects of the administered antioxidants on the cholesterol metabolism. AT could protect polyunsaturated fatty acids, thereby preserving the physical properties of membranes and the environment necessary for enzymatic activities involved in fatty acid and cholesterol metabolism (13). AT supplementation produces a momentous improvement in insulin mediated glucose utilization in healthy people, type-2 diabetics and essential hypertensives (30). In addition, α-tocopherol down-regulates the expression of the cholesterol scavenger receptors (31, 32).

The results of the present study revealed that the level of GSH in D-2 group was lower than the control group. However, GSH level was high in the treated triple antioxidant group. GSH is the most prevalent low molecular weight antioxidant within cells and it protects cellular constituents from oxidative damage by reacting directly with oxidants or by acting as the substrate for glutathione peroxidase to scavenge peroxides (33). GSH also promotes the antioxidant properties of AA and α -tocopherol (34). When

the GSH molecule neutralizes the free radicals, the GSH molecule is converted to oxide form (GSSG). The GSSG is again converted to GSH use to NADPH by the GSH reductase enzyme. The conservation and formation of NADPH in the cells are by the activity of pentose-phosphate pathway and malic enzyme (35). With an insufficient insulin level, the activities of glucose-6-phosphate dehydrogenase in pentose phosphate shunt decrease and glutathione reductase led to impairment of GSH regeneration and increased the level of GSSG. Declined GSH level in the D-2 group may be due to the lack of insulin. Increased GSH level in the triple antioxidant group may be due to the consequences of antioxidants. LA in triple antioxidant mixture has important effects on the glutathione synthesis. Packer *et al* (36) suggested that LA increases the level of glutathione by facilitating the transport of cystine and increases the level of intracellular availability of cysteine. The supply of cysteine is rate limiting for *de novo* synthesis of GSH in many cell types. It has been revealed to be potent antioxidants of the LA, to regenerate through redox cycling other antioxidants like AA and α -tocopherol, and to raise intracellular GSH levels (37, 38). Clinical trials have been shown that LA improved glucose metabolism in diabetic patients and LA treatment also increased insulin sensitivity in type-2 diabetic patients and enhanced glucose transport into skeletal muscle isolated from both obese and lean Zucker rats (18, 39). Yilmaz *et al* (40) found that the level of GSH in erythrocytes of diabetic rats was increased by the combination of the LA, AA 6 palmitate. Dincer *et al* (41) reported that in the LA supplemented group, lipid peroxidation levels were decreased; GSH levels were increased in the liver and pancreas tissues. Powell *et al* (42) suggested that the level of GSH concentrations in the human intestinal epithelial cell line reverted to normal by the administration of LA. Baydas *et al* (43) found that the LA supplementation significantly prevented the increase in lipid peroxidation levels found in diabetic rats, and GSH levels were increased by the administration of LA. Paolisso *et al* (14) suggested that percentage increase in plasma AA levels was correlated with the percentage decline in plasma free radicals and increase in GSH levels. Supplementation with AA, AT and beta-carotene resulted in an improvement of the antioxidative status of kidneys of rats with STZ-induced diabetes (44).

In the FA composition of liver tissue, although palmitic acid levels in D-1 and D-2 and D+AT+AA+LA groups were higher than the control group, stearic acid levels were low in the same groups. In mammalian cells, palmitic and stearic acids are major products of *de novo* synthesis by the activity of cytoplasmic FA synthase (45). The present study showed that the level of 18:1 n-9 in fatty acid composition of liver and muscle tissues was low in the D-2 group. Desaturases are key enzymes in the biosynthesis of the monounsaturated and polyunsaturated fatty acids and thereby contribute to the control of the

fatty acid-dependent structure and disorder of the membrane. A relationship has been observed between the 16:0/16:1 and 18:0/18:1 ratios usually used as an index of *in vivo* Δ^9-desaturase activity. The significant increase in the proportions of 16:1, n-7 and 18:1, n-9 may reflect higher activity of Δ^9 desaturase in the cell. An upsurge in the Δ^9 desaturase activity has been reported to be related to hyperinsulinemia. It has been found to induce an over-expression of Δ^9 desaturase by the effect of insulin in animals (45-47). The level of 18:1 in both liver and muscle tissues of insulin treated diabetic groups was higher than the D-2 group. This increase may be due to the effects of external insulin injection during diabetes duration. Doullet and Ciavatti (4) found that the levels of 16:1 n-9 are induced by diabetes in liver, aorta and plasma. The regulation of Δ^9 desaturase is important as physiological and its activity is sensitive to dietary changes, hormonal imbalance etc. (48).

Lipid peroxidation is a significant aspect of oxidative damage and oxidative modification of phospholipid polyunsaturated fatty acid residues in membrane structure (49). In the current study, while the 18:2, n-6 level in both liver and muscle tissues was high in the D-1, 2 and D+AT+AA+LA groups, arachidonic acid (20:4, n-6) level was low in the D-2 and D+AT+AA+LA groups. However, DHA level was high in the D-2 group. Brenner (50) found the opposite result, and reported that the proportion of this fatty acid increased in all those tissues in insulin-dependent diabetes. In addition, Hu *et al* (51) revealed that the progression of the diabetes mellitus type-1 increased mainly the DHA in the phosphatidylcholine and phosphatidylethanolamine of rat liver tissues. Prolonged diabetes causes profound alterations in the fatty acid composition of phospholipids in liver, heart, kidney, testis, spleen and brain tissues. In poorly controlled diabetes the relative level of 20:4, n-6 decrease and increase in that of LA (18:2). $\Delta^{6/5}$ desaturases are key enzymes for the synthesis of arachidonic acid and DHA in mammals and they are insulin dependent (6, 50). It has been revealed that the depressed Δ^6 desaturase is restored by insulin stimulation of the gene expression of its mRNA in experimental type-1 diabetes. 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 in almost all the tissues (50).

Skeletal muscle is a major site of insulin action, and insulin sensitivity may be related to the fatty acid composition of muscle lipids. Mohan *et al* (53) reported that the activities of $\Delta^{6\ 5}$ desaturases are depressed in experimental diabetes and in humans with insulin- and non-insulin-dependent diabetes. Doullet and Ciavatti (11) suggested that the α-tocopherol partially corrected Δ^9 desaturase activity and consequently enhanced monounsaturated acids in all tissues of experimental diabetes. In the present results, treatment of triple antioxidants to

diabetic groups increased the level of monounsaturated fatty acids. This increase might be due to the effects of AT, AA, LA and insulin administration.

Antioxidant therapy alone had no effect on the measured parameters in either the diabetic or control animals. However, combined insulin and antioxidant therapies normalized blood pressure, plasma MDA and urinary protein in the diabetic animals (25). Insulin therapy alone resulted in incomplete reduction of plasma MDA and blood pressure. Antioxidant therapy was ineffective when given alone, but when combined with insulin treatment, resulted it normalized plasma MDA, blood pressure and reduced urinary protein excretion (54). Although antioxidant treatments can illustrate benefits in animal models of diabetes, evidence from large clinical trials suggests that new and more powerful antioxidants need to be studied to demonstrate whether antioxidants can be effective in treating complications (55).

Conclusion

In conclusion, it was observed that GSH levels in liver and muscle tissue of diabetic and non-diabetic rats were increased by triple antioxidant mixture; in particular, the administration of LA increased the level of GSH in non-diabetic rats. In addition, the administration of triple antioxidant mixture and insulin were increased the level of products the SCD in liver tissue.

Acknowledgment

References

1. Berdanier AD. Diabetes and nutrition: the mitochondrial part. J Nutr 2001; 131:344– 353.
2. Maritim AC, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: A review. J Biochemical Mol Toxicol 2003; 17:24-38.
3. Emilien G, Maloteaux JM , Ponchon M. Pharmacological management of diabetes: recent progress and future perspective of daily drug treatment. Pharmacol Ther 1999; 81: 37-51.
4. Engelgau MM, Geiss LS. The burden of diabetes mellitus. In: Leahy JL, Clark NG, Cefalu WT. editors. Medical Management of Diabetes Mellitus. Mark Dekker, New York: 2000.p.1–17.
5. Biessels GJ, Smale S, Simone EJ, Kamal DE, Gispen WH. The effect of gamma-linolenic acid–alpha-lipoic acid on functional deficits in the peripheral and central nervous system of streptozotocin-diabetic rats. J Neurol Sci 2001; 182:99–106.
6. Shin CS, Lee MK, Park KS, Kim SY, Cho BY, Lee HK, *et al.* Insulin restores fatty acid composition earlier in liver microsomes than erythrocyte membranes in streptozotocin-induced diabetic rats. Diabetes Res Clin Pract 1995; 29:93-98.
7. Van BilsenM, DeVries JE, VanderVusse GJ. Long-term effects of fatty acids on cell viability and gene expression of neonatal cardiac myocytes. Prostaglandins Leukot Essent Fatty Acids 1997; 57: 39-45.
8. Van der Vusse GJ, Glatz JFC, Van Nieuwenhoven FA, Reneman RS, Bassingthwaighte JB. Transport of long-chain fatty acids across the muscular endothelium. Adv Exp Med Biol 1998; 441: 181-191.
9. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campel LV. The relation between insulin sensitivity and the fatty acid composition of skeletal muscle. New Eng J Med 1993; 328:238-244.
10. Fla´via RC, Seelaender MCL. Liver denervation affects hepatocyte mitochondrial fatty transport capacity. Cell Biochem Funct 2004; 22:9–17.
11. Poisson JPG, Cunnane SC. Long-chain fatty acid metabolim in fasting and diabetes-relation between altered desaturase activity and fatty-acid composition. J Nutr Biochem 1991; 2:60-70.
12. Mimouni V, Poisson JP. Altered desaturase activities and fatty acid composition in liver microsomes of spontaneously diabetic Wistar BB rat. Biochim Biophys Acta 1992; 1123:296-302.
13. Douillet C, Ciavatti M. Effect of vitamin E treatment on tissue fatty acids and cholesterol content in experimental diabetes. J Nutr Biochem 1995; 29:319-326.
14. Reche P, Perham RN. Structure and selectivity in post-translational modification: attaching the biotinyl-lysine and lipoyl-lysine swinging arms in multifunctional enzymes. EMBO J 1999; 18: 2673–2682.
15. Fang YZ, Yang S, Wu GY. Free radicals, antioxidants, and nutrition Nutrition 2002;18:872-879.
16. Jacob S, Streeper RS, Fogt DL, Hokama JY, Tritschler HJ, Dietze GJ, *et al.* The antioxidant alpha-lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. Diabetes 1996; 45:1024-1029.
17. Slater TF. Free radical mechanisms in tissue injury. Biochem J 1984; 222:1–15.
18. Fang YZ, Yang S, Wu GY. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872-879.
19. Huang HY, Appel LJ, Croft KD, Miller ER, Mori TA, Puddey IB. Effects of vitamin C and vitamin E on *in vivo* lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 2002; 76:549-555.
20. Orzechowski A. Dietary prophylaxis as an alternative method for the prevention of the development of insulin resistance. Med Weter 2003; 59:767-772.
21. Folch JM, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226:497-509.
22. Christie WW. Gas Chromatography and Lipids. Glaskow: The Oil Press; 1992.p.302.
23. Katsanidus E, Addis PB. Novel HPLC analysis of tocopherols, tocotrienols, and cholesterol in tissue. Free Rad Biol Med 1999; 27:1137–1140.
24. Bragagnolo N, Rodriguez-Amaya DB. Comparison of the cholesterol content of Brazilian chicken and quail eggs. J Food Comp Analysis ?? 2003; 16: 147-153.
25. Serada TJ, Mant CT, Hodges RS. Use of sodium perchlorate at low pH for peptide separatios by reversed-phase liquid chromatography. Influence of perchlorate ion on apparent hydrophilicity of positively charged amino acid side-chains. J Chromatogr 1997; 776:153-165.
26. Yoshida T. Determination of reduced and oxidized glutathione in erythrocytes by high performance liquid chromatography with ultraviolet absorbance dedection. J Chromatogr B 1996; 678: 157-168.
27. Gallou G, Ruelland A, Legras B, Maugendre D, Allanic H, Cloarec L. Plasma malondialdehyde in type 1 and type 2 diabetic patients. Clin Chim Acta 1993; 214:227–234.
28. Mero N, Syvanne M, Taskinen MR. Postprandial lipid metabolism in diabetes. Atherosclerosis 1998; 141:53-55.
29. Steinberg HO, Baron AD. Vascular function, insulin resistance and fatty acids. Diabetologia 2002; 45:623–634.
30. Paolisso G, D'Amore A, Giugliano D, Ceriello A, Varricchio M, D'Onofrio F. Pharmacologic doses of vitamin E improve insulin action in healthy subjects and non-insulin-dependent diabetic patients. Am J Clin Nutr 1993; 57:650–656.
31. Teupser D, Thiery J, Seidel D. Alpha-tocopherol down-regulates scavenger receptor activity in macrophages. Atherosclerosis

1999; 144:109–115.

32. Ricciarelli R, Zingg JM, Azzi A. Vitamin E reduces the uptake of oxidized LDL by inhibiting CD36 scavenger receptor expression in cultured aortic smooth muscle cells. Circulation 2000; 102: 82–87.

33. Beutler E. Nutritional and metabolic aspects of glutathione. Ann Rew Nutr 1989; 9:287-302.

34. May JM, Qu ZC, Whitesell RR, Cobb CE. Ascorbate recycling in human erythrocytes: Role of GSH in reducing dehydroascorbate. Free Rad Biol Med 1996; 20:543-551.

35. Sies H. Glutathione and its role in cellular functions. Free Rad Biol Med 1999; 27:916–921.

36. Packer L, Witt EH, Tritschler HJ. Alpha-Lipoic acid as a biological antioxidant. Free Rad Biol Med 1995; 19:227-250.

37. Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. Free Rad Biol Med 1997; 22:359-378.

38. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition 2001; 17:888–895.

39. Jacob S, Ruus P, Hermann R, Tritschler HJ, Maerker E, Renn W, *et al.* Oral administration of RAC-alphalipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: a placebo-controlled pilot trial. Free Rad Biol Med 1999; 27:309–314.

40. Yilmaz O, Ozkan Y, Yıldırım M, Ozturk AI, Ersan Y. Effects of alpha lipoic acid, ascorbic acid-6-palmitate and fish oil on the glutathione, malonaldehyde and fatty acid levels in erythrocytes of streptozotocin induced diabetic male rats. J Cell Biochem 2002; 86:530-539.

41. Dincer Y, Telci A, Kayali R, Yilmaz IA, Cakatay U, Akcay T. Effect of alpha-lipoic acid on lipid peroxidation and anti-oxidant enzyme activities in diabetic rats. Clin Exp Pharm Physiol 2002; 29: 281-284.

42. Powell LA, Warpeha KM, Xu WM, Walker B, Trimble ER. High glucose decreases intracellular glutathione concentrations and upregulates inducible nitric oxide synthase gene expression in intestinal epithelial cells. J Mol Endocrinol 2004; 33:797-803.

43. Baydas G, Donder E, Kiliboz M, Sonkaya E, Tuzcu M, Yasar A, *et al.* Neuroprotection by alpha-lipoic acid in streptozotocin-induced diabetes. Biochem-Moscow 2004; 69:1001-1005.

44. Mekinova D, Chorvathova V, Volkovova K, Staruchova M, Grancicova E, Klvanova J, *et al.* Effect of intake of exogenous vitamin C, Vitamin E and beta carotene on the antioxidative status in kidneys of rats with streptozotocin-induced diabetes. Nahrung 1995; 39:257-261.

45. Leonardo AE, Pereiraa SL, Sprecherb H, Huangam YS. Elongation of long-chain fatty acids. Prog Lipid Res 2004; 43:36–54.

46. Jones BH, Standridge MK, Claycombe KJ, Smith PJ, Moustaı̈d-Moussa N. Glucose induces expression of stearoyl-CoA desaturase in 3T3–L1 adipocytes. Biochem J 1998; 335:405–408.

47. Legrand P, Bensadoun A. Stearyl-CoA desaturase activity in cultured rat hepatocytes. Biochim Biophys Acta 1991; 1086:89–94.

48. Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res 1999; 40:1549-1558.

49. Quartacci MF, Cosi E, Navari-Izzo F. Lipids and NADPH dependent superoxide production in plasma membrane vesicles from roots of wheat grown in excess copper. J Exp Bot 2000; 52:77–84.

50. Brenner RR, Bernasconi AM, Garda HA. Effect of experimental diabetes on the fatty acid composition, molecular species of phosphatidyl-choline and physical properties of hepatic microsomal membranes. Prostaglandins Leukot Essent Fatty Acids 2000; 63:167-176.

51. Hu QZ, Ishii E, Nakawa Y. Differential changes in relative levels of arachidonic acid in major phospholipids from rat tissues during the progression of diabtestes. J Biochem 1994; 115:405-408.

52. Mohan IK, Das UN. Effect of L-arginine-nitric oxide system on the metabolism of essential fatty acids in chemical-induced diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids 2000; 62: 35-46.

53. Bassi A, Avogaro A, Crepaldi C, Pavan P, Zambon S, Marin R, *et al.* Short-term diabetic ketosis alters n-6 polyunsaturated fatty acid content in plasma phospholipids. J Clin Endocronol Metabol 1996; 81:1650-1653.

54. Koo JR, Ni ZM, Oviesi F, Vaziri ND. Antioxidant therapy potentiates antihypertensive action of insulin in diabetic rats. Clin Exp Hypertens 2002; 24:333-344.

55. Scott JA, King GL. Title: Oxidative stress and antioxidant treatment in diabetes. Vitamin E & Health Annals. N Y Acad Sci 2004; 1031:204-213.