Lipoprotein Oxidizability and Antioxidant Activity In Type 1 Diabetics: Relation to Duration, Glycemic Control and Hyperketonemia, and the Effect of Vitamin E Supplementation

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Abstract:

Among many hypotheses, oxidizability of lipoproteins has been implicated in the hyperlipidemia and the development of atherosclerosis and cardiovascular disease in diabetics as well as in the general population. Children with type 1 diabetes are particularly at high risk of early development of atherosclerosis. Studies evaluating LDL oxidizability in type 1 diabetics reported conflicting results and the benefit of antioxidants supplementation is still debatable. The present study included 70 (29 males, 41 females) children and adolescents with type 1 diabetes, attending the Diabetic Endocrine Metabolic Pediatric Unit at Children's Hospital of Cairo University, who were investigated and compared to a control group of 36 (18 males, 18 females) healthy children and adolescents regarding lipid profile, susceptibility of LDL to in vitro oxidation as well as antioxidant activity. Special emphasis was done on the relation of increased lipid peroxidation to diabetes duration, glycemic control and hyperketonemia. The benefit of vitamin E supplementation (400 IU/day) was tested in 46/70 type 1 diabetics regarding lipid peroxidation, lipid profile and glycosylation. Results revealed significantly higher mean levels of fasting blood glucose, glycated hemoglobin, acetoacetate, β-hydroxybuterate, total cholesterol, triglycerides, LDL and phospholipids in diabetics compared to controls. Diabetics also showed significantly increased oxidative stress (LDL oxidizability and serum malondialdehyde) as well as significantly lower antioxidant profile (reduced glutathione, serum vitamin E and vitamin E/cholesterol). While hyperlipidemia increased significantly with diabetes duration, the increased LDL oxidizability and decreased antioxidant activity did not show significant difference with the diabetes duration, and LDL oxidizability didn't show correlation with diabetes duration or glycated hemoglobin level. On the other hand, diabetics with subclinical hyperketonemia, showed significantly increased oxidative stress (LDL oxidizability and serum malondialdehyde) compared to the normoketonemic, and LDL oxidizability correlated significantly with acetoacetate levels in diabetics. Vitamin E supplementation (400 IU/ for 3 months) achieved a significant decrease in glycosylation with improvement of HbA_1 in 30/46 diabetics, a significant improvement of lipid profile, as well as a significant reduction in LDL oxidizability, and serum malondialdehyde, with a significant increase in reduced glutathione and vitamin E levels. There was also a significant increase in vitamin E/cholesterol and HDL/cholesterol indicating cardiovascular protection.

Conclusion: ***Type 1 diabetics are at risk of hyperlipidemia with increased LDL oxidizability and reduced antioxidant activity. LDL oxidizability and antioxidant activity were not related to the duration of diabetes or the level of glycated hemoglobin, but were significantly related to increased serum acetoacetate levels. Hyperketonemic type 1 diabetics appear to be at higher risk of increased LDL oxidizability and reduced antioxidant activity. Vitamin E supplementation in a dose of 400 IU given daily for 3 months was beneficial with improvement of glycated hemoglobin and lipid profile, reduced oxidative stress, and increased antioxidant activity.***

Introduction:

Type1 diabetes usually begins in childhood with a lifelong tendency for hyperglycemia and a high risk for developing serious complications. Accelerated atherosclerotic disease has been observed and reported in type 1 diabetics, being 2-4 times the risk in non-diabetics, with increased morbidity and mortality.[1,2]

It is now known that atherosclerosis is not a disease affecting elderly people but starts as early as in childhood. This makes children with diabetes particularly prone to early development of atherosclerosis.[1,3]

The mechanisms underlying the accelerated atherosclerosis in diabetics are not fully understood. Although diabetes is frequently associated with

increased levels of coronary heart disease risk factors, these do not fully explain the accelerated development of atherosclerosis.[1] The possibility that other less well-recognized cardiovascular risk factors, such as increased LDL oxidation, may enhance the atherogenic process in individuals with diabetes has been postulated.[4-6] It has been explained that oxidized low-density lipoprotein is no longer recognized by the LDL receptor and results in intracellular accumulation of cholesterol.[1,4,5] Oxidized LDL is cytotoxic, and is likely to be responsible for foam cell necrosis and the development of an extracellular lipid core and atherosclerotic lesions.[1,6] A number of studies have suggested that elevated blood lipid peroxidation is a factor in the development of cellular dysfunction, hypercoagulability of blood, hypertension and cardiovascular disease in diabetes.[4,7-9] It is suggested that lipid oxidation could play an important role in microvascular as well as macrovascular complications of diabetes,[7,10] and many reactions associated with hyperglycemia (glucose auto-oxidation, protein glycation, etc.) may acutely and chronically increase the production of free radicals, resulting in an oxidant /antioxidant unbalance.[7,10] However, studies evaluating LDL oxidizability in type 1 diabetics reported conflicting results. Whereas some studies[5,8,9,11] reported that LDL from diabetic subjects is more susceptible to in vitro oxidation than LDL from non-diabetic subjects; other studies[12-14] reported that there was no difference between type 1 diabetics and controls in the susceptibility of LDL to oxidation. Several studies demonstrated a decrease in the occurrence of complications after supplementation with different antioxidants in a diabetic animal model, thus provide evidence for the role of lipid peroxidation damage in the development of diabetic complications.[6,7] Also, antioxidants supplementation was shown to be particularly beneficial in reducing lipid peroxidation and complications in type 1 diabetics.[6,15]

The aim of the present work was to investigate type 1 diabetic children and adolescents compared to age and sex matched controls regarding lipid profile, susceptibility of LDL to in vitro oxidation, effect of diabetes duration, glycemic control and hyperketonemia, on lipid peroxidation; and the benefit of vitamin E supplementation on plasma lipid peroxidation, lipid profile and glycated hemoglobin in an attempt to improve the outcome of diabetes.

Subjects and Methods:

The present study included 70 (29 males, 41 females) type 1diabetic children and adolescents, attending the Diabetic Endocrine Metabolic Pediatric Unit at Children's Hospital of Cairo University, and a control group of 36 (18 males, 18 females) healthy children and adolescents who were recruited from volunteering children of employees at the National Research Center (NRC) and had no family history of diabetes. All children, diabetics and control, were of the same socioeconomic status.

Clinical Evaluation:

All diabetics were initially examined and their history reviewed to exclude the presence of clinical symptoms or signs of diabetes complications, and to exclude any recent event of ketosis. Screening for subclinical microvascular complications (nephropathy, retinopathy and neuropathy) was also done to exclude their presence.

Blood Sampling:

All recruited children, diabetics and control, were asked to come for blood sampling at 8-9 am after an overnight fast of at least 12 hours. Five ml of blood were withdrawn from each child and each sample was divided into 3 tubes. The first tube, 0.5 ml, was immediately deproteinized with ice-cold 0.6 mol/L percholeric acid (1: 2), centrifuged at 3000 xg for 10 minutes and supernatant was frozen until used for the analysis of acetoacetate and β-hydroxybuterate. The second tube was centrifuged at 5000 rpm for 5 minutes and separated serum was used to measure fasting blood glucose, total cholesterol, HDL-c, triglycerides (TG), phospholipids and vitamin E. The third tube contained EDTA as an anticoagulant, with which the blood was mixed well, then the whole blood was used to measure glycated hemoglobin (HbA_1), reduced glutathione (GSH) and the remaining blood was centrifuged at 1500 xg for 15 minutes and the separated plasma was used to measure LDL-oxidizability and malondialdehyde. Sampling in diabetics was done pre and post- vitamin E supplementation.

Laboratory Methods:

Fasting blood glucose, total cholesterol, LDL, HDL and triglycerides were determined using Chemistry Analyzer Olympus AU 400. Phospholipids were determined using the kit produced by Audit Diagnostics. CAT.NO.AD312PL/SP. Acetoacetate and β-hydroxybuterate were determined using the centrifugal analyzer. Glycated hemoglobin was determined by the glycohemoglobin Kit produced by Stanbio Laboratory CAT.NO. 0350-060. Normal HbA_1 for the kit is <8%. Reduced glutathione was measured using the method of Beutler et al.,[16] serum vitamin E using the method of Nierenberg and Lester,[17] and malondialdehyde using the method of Fukunaga et al.[18] LDL oxidizability was estimated in vitro using the method of Anqi Zhang et al.[19]

For the purpose of comparison and statistical analysis, diabetics with total ketone bodies > 1 mmol/L were

considered hyperketonemic and those with total ketone bodies ≤ 1 mmol/L were considered normoketonemic, as described by Jain et al.[20]

Statistical Methods:

The statistical package for social science (SPSS) was used for data management and analysis. Quantitative data were summarized as mean and standard deviation. Comparison between two groups was performed using Student's t test, and comparison between means of more than two groups were performed using one-way analysis of variance (ANOVA). The degree of association between the different measurements was measured by Pearson's correlation coefficient; and p values ≤ 0.05 were considered significant.[21]

Results:

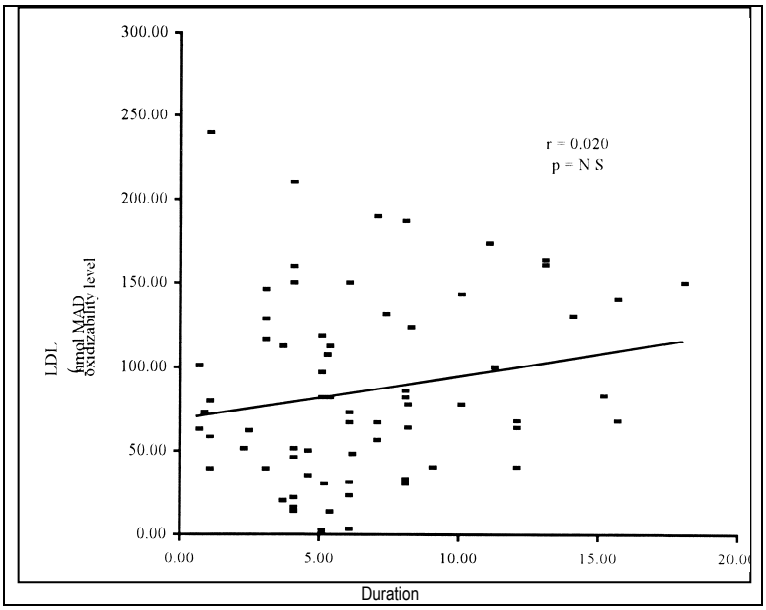

Fig.1: Correlation between the oxidizability of LDL-cholesterol and the duration of diabetes

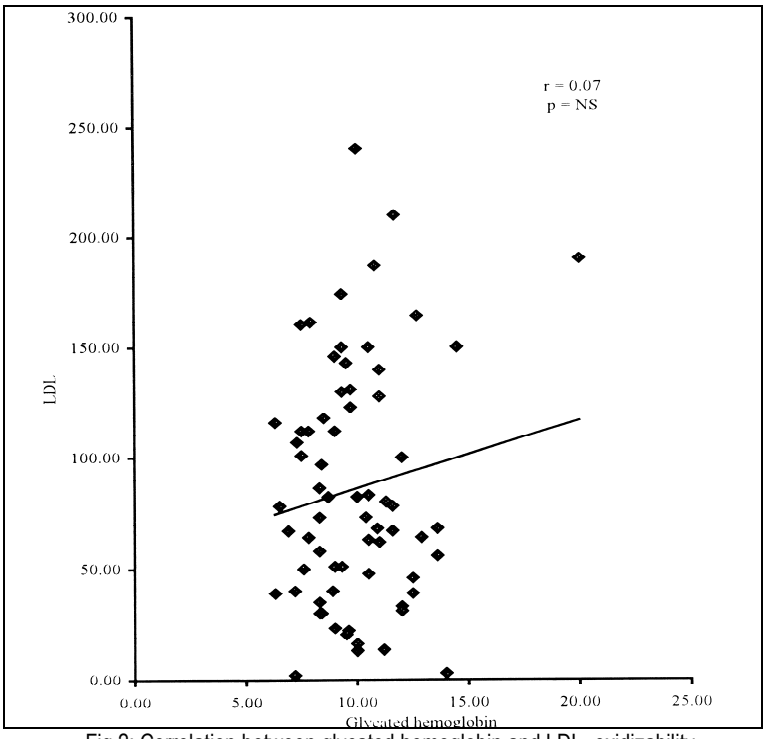

Fig.2: Correlation between glycated hemoglobin and LDL- oxidizability in diabetic children

The present study included 2 parts, a cross-sectional part and a longitudinal part. In the cross-sectional part 70 children and adolescents (29 males and 41 females) with type1 diabetes, attending the Diabetic Endocrine and Metabolic Pediatric Unit (DEMPU) of Children's Hospital at Cairo University, were investigated and compared to 36 (18 males and 18 females) age matched control subjects. Cross-sectional comparison and statistical analysis included indices of glycemic control (fasting blood glucose, glycated hemoglobin, acetoacetate and β-hydroxybuterate levels), lipid profile (total cholesterol, LDL, HDL, triglycerides and phospholipids) and lipid risk ratios (HDL/cholesterol, LDL/HDL and TG/ HDL), indicators of oxidative stress (in vitro LDL oxidizability and malondialdehyde (MDA) level), and antioxidant profile (levels of reduced glutathione (GSH), vitamin E, and vitamin E/cholestrol). Cross-sectional study also included comparison of diabetics regarding previously mentioned parameters in relation to diabetes duration and serum level of total ketone bodies. The longitudinal part of the study was a clinical trial in which the effect of regular vitamin E supplementation (400 IU/ day) for 3 months was tested in 46 type 1diabetics who agreed to take vitamin E supplementation.

Cross-sectional analysis revealed that diabetics, compared to controls, had significantly higher mean serum levels of fasting blood glucose (FBG), glycated hemoglobin, acetoacetate and β-hydroxybuterate, with (60%) having their HbA_1 above normal (>8%).

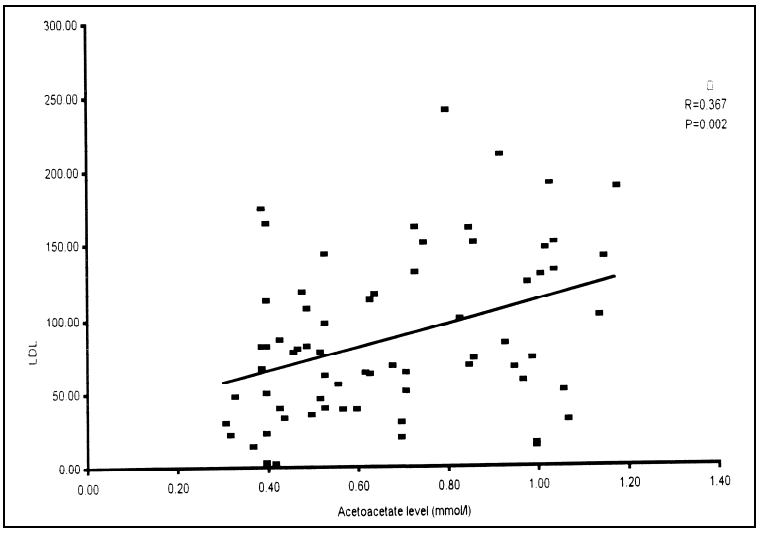

Fig.3: Correlation between acetoacetate level and LDL-oxidizability level in diabetic children

Also, significant hyperlipidemia was found in diabetics with significantly higher mean total cholesterol, LDL-cholesterol, triglycerides, phospholipids, LDL/HDL and TG/HDL; but within normal HDL and HDL/cholesterol (table I). They also had significantly higher susceptibility of LDL to in vitro oxidation and significantly lower antioxidant activity (table II). The hyperlipidemia in diabetics was significantly more in those with diabetes duration more than 10 years, but parameters of oxidative stress and antioxidant activity didn't show difference with diabetes duration (table III); and no correlation was found between LDL oxidizability and diabetes duration (figure 1).

Similarly, no correlation was found between LDL oxidizability and the mean level of glycated hemoglobin in the group of diabetics studied (figure 2).

The group of diabetics studied (n= 70) was divided into 2 groups according to the level of their total ketone bodies (acetoacetate and β-hydroxybuterate). Those with total ketone bodies > 1 mmol/L were considered as hyperketonemic and those with total ketone bodies ≤ 1 mmol/L were considered normoketonemic.. The hyperketonemic group had significantly higher mean serum acetoacetate level but comparable mean fasting blood glucose, HbA_1 and β-hydroxybuterate levels. Comparison revealed significantly higher parameters of oxidative stress in the hyperketonemic group but parameters of antioxidant activity were comparable between the 2 groups (table IV). Also, in vitro LDL oxidizability correlated significantly with serum acetoacetate levels in the group of diabetics studied (figure 3).

Forty-six out of the 70 type 1 diabetics studied completed regular vitamin E supplementation (400 IU/day) for 3 months. They included 18 males and 28 females and their age ranged from 9.0 to 18.0 years (mean ± SD 13.80± 2.5 yrs), and their diabetes duration ranged from 0.60 to 15.6 years (6.60 ± 4.2 yrs). Table V shows improvement post-supplementation in variable parameters including parameters of oxidative stress and antioxidant activity.

Table I. Comparison of glycemic indices and lipid profile in diabetics and control

Parameters	Diabetics Mean ±SD (Range)	Control Mean ±SD (Range)	t	p
M/F	29/41	18/18	/	/
Age (yr.)	13.99 ± 2.94 (8.0-18.0)	12.80 ± 3.60 (7.0-18.0)	/	/
Duration of DM (yr.)	6.44 ± 4.08 (0.6-15.60)	/	/	/
FBG (mmol/L)	15.00 ± 7.22 (3.83-31.6)	5.15 ± 0.44 (4.44-6.15)	8.19	0.000
HbA_1 (%)	9.88 ± 2.29 (6.3-14.5)	6.07± 0.44 (5.0-7.0)	9.86	0.000
Acetoacetate (mmol/L)	0.66 ± 0.25 (0.30-1.17)	0.11± 0.01 (0.09-0.14)	12.67	0.000
β-hydroxybuterate (mmol/L)	0.31± 0.06 (0.19-0.41)	0.09 ± 0.02 (0.07-0.16)	20.3	0.000
Cholesterol (mmol/L)	4.95± 1.23 (3.30-8.58)	4.39 ± 0.67 (3.62-6.54)	2.5	0.012
LDL (mmol/L)	2.76± 0.90 (1.22—5.65)	2.42 ± 0.50 (1.83-4.33)	2.07	0.04
HDL (mmol/L)	1.61± 0.31 (1.0-2.37)	1.59 ± 0.33 (1.11-2.35)	0.25	N.S.
Triglycerides (mmol/L)	1.12 ± 0.54 (0.48-3.40)	0.79 ± 0.34 (0.4-1.94)	3.33	0.001
Phospholipids (mmol/L)	2.98 ± 0.66 (1.77-5.02)	2.40 ± 0.63 (1.51-4.34)	4.36	0.000
HDL/cholesterol	0.34± 0.09 (0.16-0.70)	0.36 ± 0.06 (0.25-0.49)	- 1.19	N.S.
LDL/HDL	2.9± 0.74 (0.66-4.36)	1.62 ± 0.49 (1.0-2.49)	1.49	0.001
TG/HDL	0.72 ± 0.38 (0.20-2.29)	0.52 ± 0.26 (0.23-1.48)	2.7	0.006

Table II. Comparison of LDL oxidizability, malondialdehyde, reduced glutathione and vitamin E in diabetics and control

Parameters	Diabetics Mean ±SD (Range)	Control Mean ±SD (Range)	t	p
LDL oxidizability (nmol MDA/mg LDL)	85.47 ±53.95 (13-240)	53.79 ±13.23 (12-83)	3.46	0.001
MDA (nmol/L)	0.64±0.28 (0.29-1.78)	0.39 ±0.12 (0.27-0.85)	5.13	0.000
GSH (mg/dl)	55.5 ±19.42 (8.2-91.6)	69.1±11.83 (40-89.7)	-3.84	0.000
Vitamin E (umol/L)	10.91±5.67 (3.01-26)	14.9 ±5.5 (5.25-27.5)	-3.45	0.001
Vitamin E/ Cholesterol	2.28 ±1.30 (0.51-9.70)	3.37±1.42 (1.03-7.0)	-3.9	0.000

Table III. Lipid profile, oxidation and antioxidant activity parameters according to duration of diabetes

Parameters	≤ 1 yr Mean ±SD (Range)	>1-≤ 5 yrs Mean ±SD (Range)	>5-≤ 10 yrs Mean ±SD (Range)	> 10 yrs Mean ±SD (Range)	f	p
Cholesterol (mmol/L)	4.38 ±1.04 ***a*** (3.54-6.51)	4.7±1.16 ***a*** (3.30-8.24)	4.75±0.99***a*** (3.30-7.13)	6.01±1.50 ***b*** (4.03-8.58)	4.48	0.006
Triglycerides (mmol/L)	0.88±0.27***a*** (0.57-1.34)	1.10±0.39 ***a*** (0.66-2.27)	0.99±0.57 ***a*** (0.48-2.31)	1.61±0.75 ***b*** (0.88-3.40)	4.88	0.004
HDL (mmol/L)	1.63±0.4***a*** (1.16-2.35)	1.52±0.24 ***a*** (1.08-2.01)	1.61±0.32 ***a*** (1.0-2.37)	1.79±0.31 ***a*** (1.16-2.19)	2.11	N.S.
LDL (mmol/L)	2.35±0.80 ***a*** (1.62-3.90)	2.48±0.69 ***a*** (1.22-4.29)	2.7±0.8 ***a*** (1.55-4.53)	3.57±1.14 ***b*** (2.01-5.65)	5.32	0.002
Phospholipid (mmol/L)	2.88±0.72 ***a*** (1.77-3.89)	2.92±0.64 ***a*** (2.0-4.91)	2.86±0.46 ***a*** (2.02-3.84)	3.41±0.89 ***a*** (2.45-5.0)	2.24	N.S.
LDL oxidizability	81.88±70.5 ***a*** (39.0-240)	79.94±56 ***a*** (16.0-210)	78.8±50.3 ***a*** (13.0-190)	111.8±46 ***a*** (40.0-174)	1.17	N.S.
MDA (nmol/L)	0.73±0.19 ***a*** (0.49-1.03)	0.56±0.19 ***a*** (0.36-1.10)	0.64± 0.29 ***a*** (0.36-1.70)	0.71±0.41 ***a*** (0.29-1.78)	1.13	N.S.
GSH (mg/dl)	45.39±19 ***a*** (22.5-55)	57.29±22 ***a*** (8.20-86.3)	57.36±18.8 ***a*** (22.0-91.0)	60±11.5 ***a*** (38.0-72.0)	1.08	N.S.
Vitamin E (umol/L)	8.73±4.87 ***a*** (3.0-10.72)	10.3±4.9 ***a*** (3.50-20.2)	11.57±6.3 ***a*** (3.32-32.0)	13.19±5.4 ***a*** (7.0-25.5)	1.21	N.S.
Vitamin E/ cholesterol	1.68± 0.77 ***a*** (0.73-2.69)	2.17 ±1.1 ***a*** (0.72-2.71)	2.53±1.69 ***a*** (0.80-3.91)	2.25±0.78 ***a*** (0.82-3.72)	0.88	N.S.

a,b Letters not shared on each horizontal column show significance (p≤ 0.05) as analyzed by student's t test.

Table IV. Comparison between hyper-and normo-ketonemic diabetics

Parameters	Hyper-ketonemia (> 1 mmol/L) n = 31 Mean ±SD (Range)	Normo-ketonemia (≤ 1 mmol/L) n = 39 Mean ±SD (Range)	t	p
FBG (mmol/L)	15.62±7.59 (3.83-30.83)	14.26±6.72 (5.77-31.6)	0.79	N.S.
HbA_1 (%)	10.12±2.48 (6.3-14.5)	9.69±2.13 (6.3-14.0)	0.78	N.S.
Acetoacetate (mmol/L)	0.90±0.15 (0.61-1.17)	0.46±0.10 (0.30-0.70)	13.77	0.000
β-hydroxybuterate (mmol/L)	0.32±0.06 (0.19-0.41)	0.30±0.06 (0.20-0.41)	1.15	N.S.
LDL oxidizability (nmol MDA/mg LDL)	111.3±57.4 (20.0-240)	64.9±41.20 (13.0-174)	3.9	0.000
MDA (nmol/L)	0.71±0.35 (0.36-1.78)	0.58±0.19 (0.29-1.10)	2.07	0.042
GSH (mg/dl)	54.99±18.7 (22.0-91.6)	55.9±20.2 (8.20-80.0)	-0.20	N.S.
Vitamin E (umol/L)	11.41±5.13 (4.64-25.05)	10.52±6.11 (3.01-32.0)	0.64	N.S.
Vitamin E /cholesterol	2.27±0.77 (0.96-4.06)	2.28±0.60 (0.51-9.70)	-0.005	N.S.

Table V. Comparison of laboratory parameters in 46 type 1 diabetics pre and post supplementation with vitamin E

Parameters	Pre-supplementation (n=46) Mean±SD (Range)	Post-supplementation (n=46) Mean±SD (Range)	t	p
FBG (mmol/L)	16.69±8.2 (4.44-33.0)	16.61±7.70 (4.11-31.0)	-1.12	N.S.
HbA_1 (%)	10.72±2.47 (6.3-14.5)	9.49±1.82 (6.4-14.4)	-2.76	0.008
Cholesterol (mmol/L)	5.4±2.05 (3.3-16.5)	4.75±1.14 (2.84-8.79)	-2.46	0.017
Triglycerides (mmol/L)	1.25±0.62 (0.57-3.40)	1.12±0.50 (0.50-2.71)	0.017	N.S.
HDL (mmol/L)	1.62±0.29 (1.0-2.2)	1.62±0.23 (1.2-2.32)	0.009	N.S.
LDL (mmol/L)	2.87±0.93 (1.22-5.16)	2.63±0.92 (1.18-5.52)	-2.07	0.009
Phospholipids (mmol/L)	3.12±0.70 (2.0-5.0)	2.82±0.52 (2.0-4.48)	-4.43	0.008
HDL/cholesterol	0.32±0.09 (0.10-0.53)	0.35±0.06 (0.25-0.49)	2.5	0.016
LDL/HDL	1.83±0.74 (0.7-4.36)	1.62±0.49 (0.85-2.66)	-1.5	NS
TG/HDL	0.79±0.45 (0.32-2.24)	0.70±0.31 (0.27-1.5)	-1.32	NS
LDL oxidizability (nmol MDA/mg LDL)	94±50.37 (13.0-190)	67.6±36.6 (16.0-178)	-3.36	0.002
MDA (nmol/L)	0.62±0.31 (0.33-1.78)	0.44±0.15 (0.30-0.88)	-4.6	0.000
GSH (mg/dl)	57.13±16.7 (22.0-91.6)	67.8± 20.3 (41.0-115)	3.81	0.000
Vitamin E (umol/L)	11.59±5.75 (3.32-25.52)	22.23±7.03 (12.6-42.0)	10.23	0.000
Vitamin E/ cholesterol	2.22±1.10 (0.52-4.76)	5.02±1.41 (2.09-8.12)	13.04	0.000

Discussion:

The most important finding in this study was that type 1 diabetics, compared to control subjects, showed increased oxidative stress with significantly higher susceptibility of LDL to in vitro peroxidation and higher level of serum malondialdehyde (a product of peroxidation); and had significantly poorer plasma antioxidant activity, with significantly lower levels of serum reduced glutathione and vitamin E. The increased oxidative stress and the decreased antioxidant profile were comparable between diabetics with variable duration, and no correlation was found between LDL oxidizability and diabetes duration or degree of glycemic control in terms of HbA_1. Our findings are similar to those reported by other studies,[8,9,11,22] which demonstrated increased oxidizability in diabetics compared to control; and in agreement with Jain et al.,[22] who found no relationship between duration of diabetes or HbA_1 with the level of in vitro oxidizability of LDL and VLDL in diabetics. However, previous data on LDL oxidizability in type 1 diabetics are generally conflicting, with studies demonstrating increased,[8,9,11,22] unchanged,[12-14] or reduced[23,24] susceptibility of plasma LDL to copper induced oxidative change in diabetics.

The clear demonstration of enhanced susceptibility to peroxidation of LDL from type 1 diabetics in the present study may be related to the fact that LDL was rapidly isolated and utilized immediately in oxidation experiments, thereby reducing auto-oxidation of samples during preparation and storage. Also, samples from diabetic and control subjects were isolated and run together to avoid a systematic bias resulting from inter-assay variation over time.

Another significant finding in the present study is that diabetics had hyperlipidemia compared to controls, significantly more in those with diabetes duration more than 10 years, indicating an increased cardiovascular risk in this group. Our results are similar to several studies,[1,2,25,26] which reported that LDL-cholesterol, triglycerides and phospholipids were significantly higher in diabetics than control subjects. We also observed that vitamin E/ cholesterol was significantly lower in diabetics, but comparable within diabetics with variable diabetes duration, similar to other studies.[22,27]

It has been previously suggested that LDL oxidative susceptibility in type 1 diabetes may be dependent on changes in LDL, triglycerides and phospholipid levels influencing lipoprotein fluidity and susceptibility to oxidative change.[28-30] Also, some investigators[8,24,31] found that normolipidemic type 1 diabetics appear to have unchanged adjusted lipoprotein vitamin E levels despite having a reduced plasma total antioxidant capacity. The group of type 1 diabetics included in the present study were hyperlipidemic which might then explain their disturbed vitamin E /cholesterol ratio.

We also observed that LDL oxidizability and serum malondialdehyde were significantly higher in diabetics with hyperketonemia (ketone bodies > 1 mmol/L) compared to those with normoketonemia ($\leq$ 1 mmol/L), and a significant positive correlation was found between serum acetoacetate levels and LDL oxidizability in diabetics. This is in agreement with other studies,[20,22,23] which demonstrated that lipoproteins of hyperketonemic diabetic patients have greater susceptibility to copper induced lipid peroxidation in vitro, and that the ketone body acetoacetate, and not ß-hydroxybuterate, can generate toxic oxygen radicals and cause lipid peroxidation. Jain and McVie[33] found that oxidative stress in RBCs exposed to elevated acetoacetate concentration is increased in vitro, and in RBCs of hyperketonemic type 1 diabetics in whom excessive acetoacetate is produced endogenously in vivo. These findings suggest that ketosis is a risk factor in the development of cellular oxidative stress in diabetes, and that it is the acetoacetate, not ß-hydroxybuterate, that causes an increase in lipid peroxidation.

It is to be noted that inspite of the increased serum acetoacetate found in the group of diabetics included in the present study, none of them had recent episodes of ketosis. This is probably because their levels of ketones were much lower than those known to occur in patients with coma and severe insulin deficiency.[33] In cases with uncontrolled diabetes, the level of ketone bodies begins to rise in the blood (ketonemia) because the body fuel is mainly derived from fat. The blood concentration of ketone bodies in diabetic patients with severe ketosis may reach more than 20 times the normal level, reaching up to 10 mmol/L in diabetics with severe ketosis compared with concentrations less than 0.5 mmol/L in normal subjects.[33,34]

The supplemented group 46 diabetics included were of variable duration and glycemic control with a mean duration of 6.6 years and a mean HbA1 of 10.7%. Data analysis after completion of vitamin E supplementation (400 IU/ for 3 months) showed no significant change in fasting blood glucose, but there was a significant decrease in glycosylation with improvement of HbA1 in 30/46 diabetics (65%). There was also significant decrease in cholesterol, LDL and phospholipids as well as significant reduction in LDL oxidizability, and serum malondialdehyde, with increase in reduced glutathione and vitamin E levels. There was also a significant increase in vitamin E/cholesterol and HDL/cholesterol indicating cardiovascular protection. The risk ratio LDL/HDL also decreased but did not reach statistical significance although LDL decreased significantly with supplementation. This is in agreement with other

studies,[15,35] which reported decreased in vitro LDL-oxidizability with regular vitamin E supplementation. Also, the clinical trial by Jain et al.[15] demonstrated that vitamin E supplementation can increase reduced glutathione and lower malondialdehyde and HbA_1 concentrations. However, a recent analysis of prospective, controlled clinical trials of vitamin E reveals conflicting results regarding the benefit of vitamin E supplementation. It seems that patients with high levels of oxidant stress or depletion of natural antioxidant defence systems may be the most likely to benefit from antioxidant therapy.[6]

Conclusion:

Type 1 diabetics are at risk of hyperlipidemia with increased LDL oxidizability and reduced antioxidant activity. While the risk of hyperlipidemia is increased with longer diabetes duration and poor glycemic control, LDL oxidizability and antioxidant activity were not related neither to the duration of diabetes nor to the glycated haemoglobin but was related to acetoacetate levels. Hyperketonemic type 1 diabetics appear to be at higher risk of increased LDL oxidizability and reduced antioxidant activity. Vitamin E supplementation in a dose of 400 IU given daily for 3 months was beneficial with improvement of glycated hemoglobin, lipid profile, oxidative stress, and antioxidant activity. A randomized controlled trial involving a larger number of diabetics is needed to verify the benefit of vitamin E supplementation.

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