Lipid peroxide levels in diabetics with micro- and macro-angiopathies

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This study examined lipid peroxides and lipid profile in diabetics with and without complications. One hundred seventeen non-insulin dependent diabetics and 34 insulin dependent diabetics were compared with healthy controls. All diabetics had poor glycemic control. Dyslipidemia was observed in diabetics, with the highest value being recorded in diabetics with complications.

Lipid peroxide values were elevated in diabetics as compared with healthy controls. The highest values were found in patients with complications. Peroxides showed a negative but significant correlation with high density lipoproteins-cholesterol fraction. Lipid peroxide values were significantly influenced by age of the patient, duration of diabetes, age at onset of diabetes, body mass index, and duration of ischemic heart disease. These results indicate that increased peroxidative stress may be an important risk factor in the development of microangiopathy and macroangiopathy. (J. Nutr. Biochem. 5:442–445, 1994.)

Keywords: lipid peroxides; non-insulin dependent diabetes; HDL-cholesterol; macroangiopathies; body mass index; microangiopathies

Introduction

Lipid peroxides have been implicated in oxidative damage to DNA, aging, carcinogenesis, and in the pathogenesis of atherosclerosis.[1] Lipid peroxides are generated though a chain reaction of peroxidation of polyunsaturated fatty acids in the cell membranes. It has been reported that diabetic patients, particularly those with angiopathy, had higher plasma lipid peroxide levels than nondiabetics.[2,3] It has been hypothesized that oxygen-free radicals may be formed by the reaction of glycated proteins with molecular oxygen in the tissues of diabetic patients, and excessive production of oxygen free radicals may contribute to the complications associated typically with diabetes. The structural changes that occur in diabetics are oxidative in nature, and oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with the development of vascular disease in diabetics.[4] The present study was undertaken to compare the lipid peroxide levels in diabetics with and without complications, related to specific factors such as type of complication, age at onset of diabetes, duration of diabetes, and the ratio of polyunsaturated to saturated fat in the diet.

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Methods and materials

One hundred fifty-one diabetic patients were recruited from the Diabetic Out-Patients Department of B.Y.L. Nair Hospital, Bombay, India. These included 86 non-insulin dependent diabetics with complications and 31 without complications. Thirty healthy, nondiabetic subjects formed the control group.

Clinical history for each patient was recorded, and grade stage of the diabetic complication was determined by a diabetologist. In addition, age; sex; height; weight; history of diabetes, essential hypertension, and atherosclerosis; duration of diabetes; complications; treatment; and dietary information regarding the type and amount of fat used were recorded.

Fasting 10 mL blood samples were drawn from the ante-cubital vein from which 8 to 9 mL were collected into vials containing 7.5% EDTA, and the remaining 1 to 2 mL was collected into fluoride tubes for blood glucose estimation. Plasma was also analyzed for triglycerides, total cholesterol, high density lipoprotein (HDL)-cholesterol, and lipid peroxides. Total cholesterol was estimated by Watson's method,[5] HDL-cholesterol by Wybenga's,[6] and triglycerides were determined by Fletcher's method.[7] The plasma lipoperoxide levels were estimated by a modification[8] of Yagi's fluorimetric method. The data were analyzed using the Statistical Package for Social Sciences (SPSS).* Comparisons of all lipid parameters were made between healthy controls and diabetics with and without complications by one-way and two-way analysis of variance.

* PC package (software from SPSS Inc., Chicago, IL, USA) at the Tata Institute of Social Sciences, Bombay, India.

Results

Plasma lipoperoxide concentrations in non-insulin dependent diabetic (NIDD) subjects were significantly higher than in controls. Further, patients with complications had higher levels compared with subjects without complications, with no significant differences in the peroxide levels between males and females *(Figure 1)*. Insulin dependent diabetic (IDD) subjects without complications had slightly higher mean lipoperoxide values than the control group. There were only two IDDM subjects with complications, hence no comparison of IDDM patients with and without complications was possible. These findings are consistent with the results of Sato et al.[2] who observed that diabetics with angiopathy had higher levels compared with diabetics without angiopathy.

It has been suggested that elevated plasma peroxides would cause an increase in the peroxide levels in the intima of the blood vessel, which could initiate atherosclerosis.

Lipid peroxides and diabetic complications

Mean lipid peroxide levels were highest in diabetics with complications of ischemic heart disease, while the lowest values were observed in the group with nephropathy followed by cerebrovascular disease. However, these differences were not statistically significant. The lack of statistical significance could be due to the small number of diabetics with macroangiopathy and microangiopathy.

As yet no critical level or cut-off point in terms of prognosis or risk for lipid peroxides has been established. Hence, comparisons between subjects with and without complications can be used as an indication of increased or decreased peroxide levels.

In the present study, both the level of ischemic heart disease ($P < 0.001$) and its duration significantly influenced lipid peroxide values ($r = 0.347$; $P < 0.001$). The duration of ischemic heart disease ranged from 3 months to 16 years, with a median value of 1 year. It was also observed that HDL-cholesterol showed a significant ($r = -0.225$, $P < 0.01$) negative correlation with the lipid peroxide level, clearly indicating that increased peroxidation has an atherogenic effect *(Figure 2)*. Other studies have indicated a significant correlation to the LDL-fraction.[9]

There is scanty evidence available about the role of the peroxides in diabetic retinopathy and nephropathy. In the case of retinopathy, it has been suggested that lipid peroxidation may represent an initiating event in the development of senile cataracts.[10] One of the breakdown products of lipid peroxidation, malondialdehyde measured as a thiobarbituric acid reactive species, has been found to be significantly elevated in cataractous lenses. Given these conditions, it is likely that increased peroxidation could cause the various conditions of diabetic retinopathy: background diabetic retinopathy, proliferative retinopathy, maculopathy, and retinal detachments.

Role of selected factors

In the present study, the level of lipid peroxide in diabetics showed a significant correlation with age of the subject ($r = 0.286$; $P < 0.001$) *(Figure 3)* as well as age at onset of diabetes ($r = 0.297$; $P < 0.001$) *(Figure 4)*. Median values and ranges for age, age at onset of diabetes, duration of diabetes, and body mass index (BMI) are shown in *Table 1*. In normal subjects, Suematsu et al.[11] observed an age-dependent increase in lipid peroxide levels, with the highest level being found in 50- to 70-year-old subjects. Further, duration of diabetes showed a significant correlation ($r = 0.137$; $P < 0.05$) with lipoperoxide values. Analysis of variance indicated that the degree of glycemic control did not significantly influence lipid peroxide values. It is likely that increased exposure, even to mild degrees of hyperglycemia,

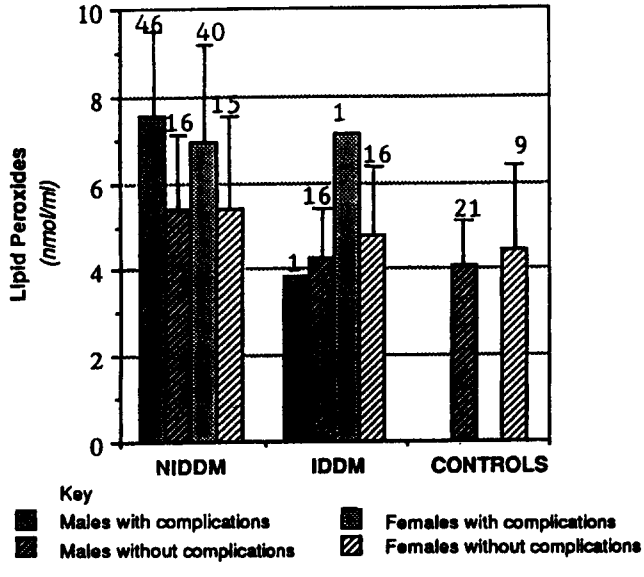

Figure 1 Lipid peroxide levels in NIDDM, IDDM, and controls. Vertical lines represent standard deviation. Numbers indicate sample size.

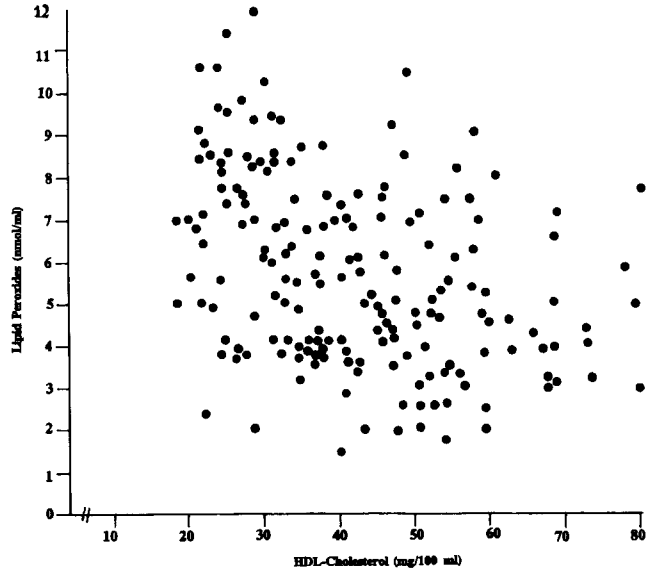

Figure 2 Lipid peroxide levels in relation to HDL cholesterol.

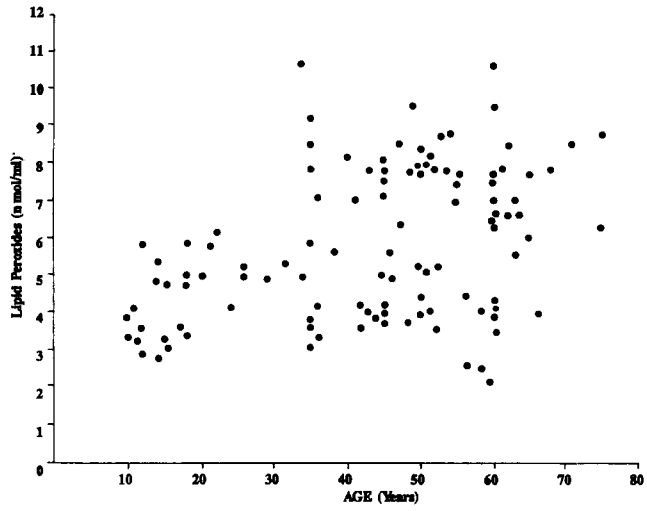

Figure 3 Lipid peroxide levels in relation to age (years).

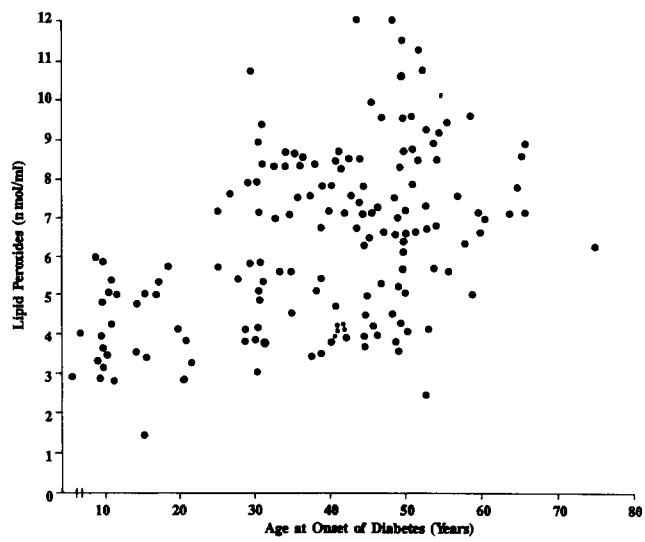

Figure 4 Lipid peroxide levels in relation to age at onset of diabetes.

Table 1 Median values and ranges for age, age at onset of diabetes, duration of diabetes, and BMI in subjects with different complications

Categories	n	Age (years)		Duration of diabetes (yrs)		Age at onset of Diabetes (years)		BMI	
		Median	Range	Median	Range	Median	Range	Median	Range
I) Non-insulin dependent diabetics with complication of:									
a) Cardiovascular disease	61	52	30 to 75	5	0.4 to 23	47	28 to 74	23.5	16.2 to 32
b) Retinopathy	14	59	35 to 68	16	0.4 to 22	43	31 to 61	22.6	15 to 28.4
c) Nephropathy	6	53	50 to 60	5	2 to 15	48	43 to 58	24.3	17.4 to 28.6
d) Neuropathy	5	50	30 to 56	2	0.5 to 6	45	30 to 54	20.7	15.6 to 22.2
II) Insulin dependent diabetics									
a) with complications	2								
b) without complications	30	19	10 to 42	7	1 to 32	13	2 to 31	19.0	15.7 to 27.1
III) Maturity onset diabetes of the young (MODY)	2								
IV) Control group-non-insulin dependent diabetics (without complications)	31	43	34 to 66	3	0.4 to 30	33	28 to 63	26	15.6 to 38.0
V) Control group-healthy non-diabetic subjects	30	40	32 to 52	—	—	—	—	22.1	16.6 to 30.4

could result in metabolic insult to the vasculature leading to increased peroxide formation.

BMI has been proposed for determining ideal body weight for height. The influence of BMI on the occurrence of specific hyperlipidemias has not been well studied. In the present study, BMI showed a weak correlation with lipid peroxide levels ($r = 0.112$; $P < 0.1$). The lipid peroxide values in these groups is shown in *Table 2*. A statistically significant difference was found between the lipid peroxide levels of the underweight and normal weight diabetics ($P < 0.01$). Thus, it seems likely that increased adiposity increases oxidative stress in diabetics.

A question of major nutritional importance is whether a relationship exists between the level of polyunsaturated fatty acids (PUFA) in the diet, the amount of dietary fat, and the generation of oxygen radicals in the body. In the present study, the total fat consumption was calculated on the basis of the 24-hour recall method. Daily total fat intakes ranged from 14.5 g to 120.5 g but did not show any significant association with the lipoperoxide values. In the present study, a very small percentage of subjects were consuming oil/fat with a high percentage of PUFA prior to and following detection of diabetes (0.7 and 4.4%, respectively). One-way analysis of variance indicated that lipid peroxide values tended to increase with the polyunsaturated to saturated ratio of the dietary fat.

Table 2 Lipid peroxide values in relation to BMI

Group	BMI Classification[12] Male	BMI Classification[12] Female	Sex	Sample Size	Lipid Peroxides (nmol/mL)
Under weight	19	19	M	17	5.48* ± 1.9†
			F	5	6.62 ± 2.8
Normal weight	19 to 25	19 to 24	M	42	6.79 ± 2.09
			F	35	5.91 ± 1.8
Over weight	25 to 27	24 to 27	M	14	6.08 ± 2.31
			F	18	6.47 ± 2.30
Obese	27	27	M	6	7.45 ± 3.61
			F	10	6.85 ± 1.8

*Mean.
†Standard deviation.

Discussion

The results of this study indicate that lipid peroxides in diabetics regardless of presence or absence of complications were higher than those found in healthy individuals. Further, factors such as duration of diabetes, age of the subjects, age at onset of diabetes, and BMI were found to be significantly correlated with lipid peroxide levels.

It is difficult to associate increased oxidative stress as a case of cause and effect relationship between the variables cited above. Whether increased oxidative stress leads to development of complications needs to be reviewed in relation to the possible causes of increased oxidative stress. Peroxides have been suggested to initiate tissue damage, and the injury to the tissue by any means increases the amount of free radicals. In diabetics, it is likely that the hyperglycemic state may provoke or enhance peroxide formation. While peroxide formation may not be accelerated in hyperglycemia, it is possible that diabetics do not have adequate capacity to withstand oxidative stress, unlike healthy individuals.

Further studies can lead to the development of effective strategies for limiting damage from increased oxidative stress or for complementing other therapeutic approaches to the treatment of diabetes.

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