EFFECT OF ω-3 FATTY ACIDS ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYME STATUS IN TYPE 2 DIABETIC PATIENTS

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SUMMARY - Background: This study was conducted to investigate the effect of ω-3 fatty acids on lipid peroxidation and antioxidant enzyme activities in non-insulin dependent diabetic patients.

Methods: Thirty-four non-insulin dependent diabetic patients were selected for this study and they were initially treated with antidiabetic drugs alone for one month. This was followed by supplementation with ω-3 fatty acids (1,080 mg of EPA and 720 mg of DHA per day) along with the antidiabetic drugs for a period of two months.

Results: No change in glycaemic control was observed in diabetic patients at the end of two months of ω-3 fatty acids therapy along with antidiabetic drugs. The combined treatment significantly reduced serum triglycerides (2.07 ± 0.94 mmol/l), before combined therapy vs 1.54 ± 0.49 mmol/l after combined therapy, P < 0.05) and increased HDL-cholesterol levels (0.93 ± 0.099 mmol/l, before combined therapy vs 1.04 ± 0.096 mmol/l after therapy, P < 0.01). The raised lipid peroxide levels (5.14 ± 0.61 µmol MDA/l in controls vs 6.36 ± 1.56 µmol MDA/l in diabetic patients, P < 0.001) were significantly decreased in these patients after the combined therapy (6.36 ± 1.56 µmol MDA/l, after combined therapy vs 5.16 ± 0.7 µmol MDA/l, after combined therapy, P < 0.01). Among the erythrocyte antioxidant enzymes, the Glutathione peroxidase activity was increased (32.5 ± 4.6 U/g Hb after combined therapy vs 42.25 ± 4.6 U/g Hb before combined therapy) while no change was observed in Catalase (99.7 ± 30.4 KU/g Hb before combined therapy vs 85.35 ± 23.41 KU/g Hb, after combined therapy) and Superoxide dismutase activities (2.5 ± 0.04 U/mg Hb/min, before therapy vs 3.01 ± 1.08 U/mg Hb/min, after combined therapy) after the 2 months of combined treatment with antidiabetic agents and ω-3 fatty acids.

Conclusion: Supplementation with ω-3 fatty acids has beneficial effects on serum triglycerides, HDL-cholesterol, lipid peroxidation and antioxidant enzymes, which may lead to decreased rate of occurrence of vascular complications in diabetes.

Key-words: type II diabetes mellitus, lipoproteins, lipid peroxides, antioxidant enzymes, ω-3 fatty acids.

RÉSUMÉ - Effet des acides gras ω-3 sur la peroxydation lipidique et le statut des enzymes antioxydantes chez les diabétiques de type 2.

Contexte : Cette étude a investigué l’effet des acides gras ω-3 sur la peroxydation lipidique et les activités enzymatiques antioxydantes chez des diabétiques non-insulino-dépendants.

Méthodes : Trente-quatre diabétiques de type 2 ont été sélectionnés et ont été initialement traités par antidiabétiques oraux pendant un mois. Une supplémentation par acides gras ω-3 a alors été instaurée (1 080 mg de EPA et 720 mg de DHA par jour) en association avec le traitement antidiabétique pendant 2 mois.

Résultats : Aucun changement du contrôle glycémique n’a été observé à la fin de cette période de 2 mois. Le traitement combiné a significativement réduit les triglycérides sériques (2,07 ± 0.94 mmol/l, avant traitement combiné vs 1,54 ± 0.49 mmol/l après traitement combiné, P < 0.05) et augmenté les taux de cholestérol-HDL (0.93 ± 0.099 mmol/l, avant traitement combiné vs 1,04 ± 0.098 mmol/l après traitement, P < 0.01). Les taux élevés de peroxydes lipidiques (5,14 ± 0.61 µmol MDA/l chez les témoins vs 6,36 ± 1,56 µmol MDA/l chez les diabétiques, P < 0,001) ont été significativement réduits chez ces patients après traitement combiné (6,36 ± 1,56 µmol MDA/l, avant traitement combiné vs 5,16 ± 0,7 µmol MDA/l, après traitement combiné, P < 0,01). Parmi les enzymes antioxydantes érythrocytaires, la Glutathion peroxidase a son activité augmentée (32,5 ± 9,9 U/g Hb/min, avant traitement combiné vs 42,25 ± 4,6 U/g Hb/min, après traitement combiné, P < 0,01) tandis qu’aucun changement n’est observé pour l’activité Catalase (99,7 ± 30,4 KU/g Hb avant traitement combiné vs 85,35 ± 23,41 KU/g Hb, après traitement combiné) ni pour l’activité Superoxide dismutase (2,6 ± 1,04 U/mg Hb/min, avant traitement combiné vs 3,01 ± 1,08 U/mg Hb/min, après traitement combiné) après 2 mois de traitement combiné par antidiabétiques et acides gras ω-3.

Conclusion : La supplémentation en acides gras ω-3 a des effets bénéfiques sur les triglycérides, le cholestérol-HDL, la peroxydation lipidique et les enzymes antioxydantes, ce qui peut contribuer à diminuer la survenue des complications vasculaires du diabète.

Mots-clés : diabète de type 2, lipoprotéines, peroxydes lipidiques, enzymes antioxydantes, acides gras ω-3.
Diabetes mellitus has been shown to be a state of increased free radical formation [1, 2]. Oxidative stress is increased in diabetes owing to increase in the production of oxygen free radicals (OFR’s) such as super oxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxide (OH$^-$) radicals and deficiency in antioxidant defense mechanisms. Auto-oxidation and non-enzymatic glycation are thought to be the reasons for increased production of OFR’s in diabetes mellitus [3, 4]. Lipid peroxidation of cellular structures, a consequence of increased OFR’s is thought to play an important role in atherosclerosis and microvascular complications of diabetes [5, 6]. Hyperlipidaemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes [7, 8]. The status of antioxidant defense mechanisms in diabetes is very contradictory as both increases and decreases of antioxidant status have been reported in diabetes [9, 10].

Omega-3 fatty acids or n-3 fatty acids and Omega-6 fatty acids or n-6 fatty acids are the essential fatty acids needed in the structure and function of cell membrane. Eicosapenta enoic acid (EPA) and Docosa hexa enoic acid (DHA) are the most predominant of ω-3 fatty acids. Numerous studies in the past have reported beneficial effects of ω-3 fatty acids in the treatment of various diseases including diabetes [11, 12]. It has been reported that these ω-3 fatty acids are useful in the treatment of hypertriglyceridemia [13, 14] and in the enhancement of HDL-cholesterol (HDL-C) levels [13] in diabetic patients. But there are no reports on the effect of ω-3 fatty acids on lipid peroxidation and antioxidant defense mechanisms in diabetic state. Hence this study was carried out to investigate the effect of ω-3 fatty acids on lipid peroxidation and antioxidant enzyme activities in diabetic patients.

**SUBJECTS AND METHODS**

Thirty-four nonobese type 2 diabetic patients who were only on oral antidiabetic drugs (27 on sulphinyl ureas, 4 on biguanides, and 3 on both) but not on any lipid lowering drugs or antioxidant therapy were recruited for this study. All the patients selected for this study were normotensive and they did not have any of the clinical complications other than diabetes. None of the patients had any diabetic complications. Forty-one age and body weight matched non-diabetic normotensive healthy subjects were also selected and treated as normal controls. Informed consent was obtained from all the study subjects. The clinical details of all the study subjects were recorded and fasting blood samples were collected and used for the measurement of blood glucose (FBG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) levels. Heparinized blood collected was used for the estimation of HbA1, lipid peroxides and erythrocyte antioxidant enzyme activities.

After the clinical and biochemical investigations all the diabetic patients selected for this study were advised diet (~ 1,800 Kcalories/day) as per their ideal body weights and they were treated with appropriate doses of oral antidiabetic drugs only for one month. Fasting blood was collected after one month of dietary control and antidiabetic treatment, for the measurement of lipid peroxides and erythrocyte antioxidant enzyme activities. After one month of antidiabetic therapy alone, these patients were then given ω-3 fatty acids of 1.080 mg of EPA and 720 mg of DHA per day in three divided doses for 2 months along with the antidiabetic treatment. The ω-3 fatty acids were given in the form of gelatinous capsules (manufactured by Merck (India) Ltd.), each capsule containing 180 mg of EPA and 120mg of DHA. Fasting blood was collected after 2 months of the combined therapy for the measurement of FBG, HbA1, serum lipids, lipoproteins and plasma lipid peroxides and erythrocyte antioxidant enzyme activities. Blood pressures were recorded before and after the combined therapy.

Another group of nonobese and normotensive type 2 diabetic patients (n = 6) were treated with oral antidiabetic drugs alone for three months. FBG, HbA1, plasma lipid peroxides and erythrocyte antioxidant enzyme activities were measured in these patients before and after one month and three months of antidiabetic therapy.

Blood glucose, serum TG, TC, HDL-C levels were measured by using standard methods [15-18]. The LDL-C and VLDL-C levels were calculated by using Friedewald formula [19]. Glycaemic control was assessed by measuring HbA1 by the procedure of Eross et al., [20]. Plasma levels of malondialdehyde (MDA), a product of lipid peroxidation was measured as TBARS by the method of Yagi et al., [21] modified by Griesmacher et al., [22]. MDA levels were measured immediately after collecting the blood samples. The percentage of recovery of MDA was 96.4%. The inter and intra assay coefficients of variation of this method were 5.2% and 3.5% respectively. In this method, addition of glucose up to 15 mM did not significantly influence the TBARS levels.

Erythrocyte catalase (CAT) activity was assayed by the method of Aebi [23], Glutathione peroxidase (GPx) activity in erythrocytes was measured by the method of Wendel [24] and Super oxide dismutase activity was measured by the method of Misra and Fridovich [25] after the removal of haemoglobin by the method of Concetti et al., [26].

**STATISTICAL ANALYSIS**

All the values were expressed as mean ± SD. Unpaired Student’s t-test was used for group comparisons.
and paired Student’s t-test was used for comparison within the same group. P values < 0.05 were considered significant.

## RESULTS

Clinical details of the study subjects are given in Table I and biochemical details at baseline and after 2 months of combined therapy are given in Table II.

The baseline FBG levels were significantly higher in all the diabetic patients compared to controls. All the diabetic patients had higher levels of HbA1 than those in controls. Serum TG, TC, LDL-C and VLDL-C were significantly higher in diabetic patients than those in normal controls. Significantly lower levels of HDL-C were observed in diabetic patients compared to controls.

There was no change in FBG and HbA1 levels in diabetic patients after 2M of treatment with antidiabetic agents and ω-3 fatty acids. They were significantly higher than those in controls.

The combined treatment in diabetic patients resulted in a significant decrease in TG levels and increase in HDL-C levels without any change in TC and LDL-C levels.

The levels of plasma TBARS and activities of erythrocyte antioxidant enzymes in the diabetic patients at baseline and after treatment with antidiabetic drugs alone and combined therapy are given in Table III. The plasma TBARS levels at baseline were

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<th>Table I. Clinical details of the study subjects.</th>
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<td><strong>Particulars</strong></td>
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<tr>
<td>Number (n)</td>
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<tr>
<td>Sex (M/F)</td>
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<td>Age (years)</td>
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<td>BMI (Kg/m²)</td>
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<td>Duration of diabetes (years)</td>
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<tr>
<th>Table II. Fasting blood glucose, HbA1, serum lipids, lipoproteins in controls, in diabetic patients before and after combined therapy.</th>
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<td><strong>Controls</strong></td>
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<td></td>
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<tr>
<td>Fasting blood glucose (mmol/l)</td>
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<tr>
<td>Glycated hemoglobin (Hb A1) (%)</td>
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<tr>
<td>Total Cholesterol (mmol/l)</td>
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<td>HDL-cholesterol (mmol/l)</td>
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<td>LDL-cholesterol (mmol/l)</td>
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<tr>
<td>VLDL cholesterol (mmol/l)</td>
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<td>Triglycerides (mmol/l)</td>
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*P < 0.001 compared to controls; **P < 0.01 compared to controls; ***P < 0.05 compared to controls; † P < 0.01 compared to baseline levels; † † P < 0.05 compared to baseline levels.
significantly higher in diabetic patients compared to those in controls.

Erythrocyte CAT activity was significantly increased, glutathione peroxidase activity was significantly decreased, and no change was observed in SOD activity in diabetic patients at baseline, compared to those in controls.

The TBARS levels were significantly decreased in diabetic patients after 1M of antidiabetic treatment, while no change was observed in the antioxidant enzyme activities after one month of antidiabetic treatment.

The TBARS levels were significantly decreased further in diabetic patients after the combined treatment compared to their levels after 1M of antidiabetic treatment alone.

Among the antioxidant enzymes, CAT and SOD did not show any significant change in their activities after 2M of combined therapy, but a significant raise in GPx activity was observed in the diabetic patients at the end of the combined treatment.

All the diabetic patients remained normotensive after the combined treatment.

Among the antioxidant enzymes, CAT and SOD did not show any significant change in their activities after 2M of combined therapy, but a significant raise in GPx activity was observed in the diabetic patients at the end of the combined treatment.

**DISCUSSION**

Omega-3 fatty acids appear likely to assume an interestingly important role in the therapeutics. They have several beneficial effects in diabetes and other diseases.

Shimura et al., [27] have reported decrease in FBG levels after ω-3 fatty acids treatment in diabetic patients and suggested that this decrease in glucose levels could be due to increased insulin sensitivity, while several other authors [14, 28] could not find any change in blood glucose levels in diabetic patients at the end of ω-3 fatty acids treatment.

In one study the HbA1 levels in diabetic patients were increased after the treatment with ω-3 fatty acids [29], but in several other studies [13, 14] there was no change in HbA1 levels in ω-3 fatty acids treated diabetic patients. In the present study also we could not find any significant change in HbA1 levels after the combined treatment. Though the mean value of HbA1 after combined therapy appears lower than that at baseline the difference between them is not statistically significant (p = 0.62) by paired Student’s t-test.

In the other group of diabetic patients, who were treated with antidiabetic drugs alone there was a significant decrease in the HbA1 levels after 3 months of the treatment.

| TABLE III. Plasma TBARS and activities of erythrocyte antioxidant enzymes in controls and diabetic patients after 1M of antidiabetic treatment alone and 2M of combined therapy. |
|-----------------|-----------------|-----------------|-----------------|
|                 | Controls        | Diabetic patients (baseline) | Diabetic patients After 1M of antidiabetic therapy alone | Diabetic patients After 2M of combined therapy |
| TBARS (µmol MDA/l) | 5.14 ± 0.61    | 6.36 ± 1.56*         | 5.62 ± 0.76 †         | 5.16 ± 0.7 †         |
| Catalase (KU/g Hb) | 85.8 ± 23.6    | 99.7 ± 30.4***       | 88.77 ± 16.26         | 85.35 ± 23.41 |
| Glutathione peroxidase (U/g Hb/min) | 40.84 ± 12.3 | 32.5 ± 9.9**       | 35.95 ± 7.04          | 42.25 ± 4.6 † |
| Superoxide dismutase (U/mg Hb/min) | 2.55 ± 0.84   | 2.6 ± 1.04           | 2.53 ± 1.1           | 3.0 ± 1.08 |

*P < 0.001 compared to controls; **P < 0.01 compared to controls; ***P < 0.05 compared to controls; † P < 0.01 compared to baseline levels; † † P < 0.05 compared to baseline levels.
Table IV. FBG, HbA1, TBARS and erythrocyte antioxidant enzyme activities in diabetic patients treated with antidiabetic treatment alone.

<table>
<thead>
<tr>
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<th>Baseline levels</th>
<th>After 1M of treatment</th>
<th>After 3M of treatment</th>
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<tbody>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>12 ± 5.6*</td>
<td>6.4 ± 0.9</td>
<td>7.05 ± 2.6</td>
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<tr>
<td>Glycated hemoglobin (HbA1) %</td>
<td>11.36 ± 2.7*</td>
<td>—</td>
<td>7.7 ± 2.1 †</td>
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<tr>
<td>TBARS (µmol MDA/l)</td>
<td>6.33 ± 0.46*</td>
<td>5.8 ± 0.47</td>
<td>5.6 ± 0.3 †</td>
</tr>
<tr>
<td>Catalase (KU/g Hb)</td>
<td>95.0 ± 14.9</td>
<td>79.4 ± 19.2</td>
<td>88.9 ± 14.0</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/g Hb/min)</td>
<td>34.7 ± 12.6</td>
<td>38.0 ± 7.36</td>
<td>38.9 ± 5.0</td>
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<tr>
<td>Superoxide dismutase (U/mg Hb/min)</td>
<td>2.18 ± 0.8</td>
<td>2.37 ± 1.1</td>
<td>2.4 ± 0.5</td>
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*P < 0.001 compared to controls; † P < 0.05 compared to baseline levels.

The serum TG levels were significantly decreased in diabetic patients after the combined treatment, which is in agreement with the observations of Mc Manus et al., [14], Fasching et al., [30] and Zak et al., [13]. Several authors [13, 31, 32] have reported decreased TC levels in the diabetic patients after treatment with ω-3 fatty acids, but in this study, the combined treatment had no effect on TC levels in diabetic patients.

Zak et al., [13] and Mc Manus et al., [14] have shown no change in LDL-C levels after treatment with ω-3 fatty acids, while Haban et al., [33] have reported increase in LDL-C levels after ω-3 fatty acid treatment, but in our study we could not find any significant change in LDL-C levels in the diabetic patients after the combined treatment. Though the mean value of LDL-C in diabetic patients after combined therapy is higher than that at baseline the difference between them is not statistically significant by paired Student’s t-test.

There are reports of both increase [13, 31] and no change [14, 30] in HDL-C levels in diabetic patients treated with ω-3 fatty acids. In this study we have observed increased HDL-C levels in diabetic patients after the combined treatment.

Lipid peroxides were measured in this study as TBARS by measuring plasma levels of malondialdehyde (MDA). Lipid hydroperoxides breakdown in the presence of iron or other metal complexes to form aldehydes, e.g. malondialdehyde. The most commonly applied test is the TBA reaction for the measurement of MDA, either directly in plasma or following hydrolysis at acid pH which produces MDA from the breakdown of lipid peroxides [46]. The term thiobarbituric acid reacting substances (TBARS) is often applied to the measurement of lipid peroxides by TBA reaction and is used interchangeably with MDA. In the given method, precautions were taken to prevent the interference of the other TBA reacting substances and to increase the specificity and sensitivity of this method [47].

Several authors [9, 22, 34, 35] have reported increased levels of lipid peroxides in diabetic patients, while a few others could not find any significant difference [5, 36] in lipid peroxide levels in diabetic patients. But in this study a significant increase in baseline lipid peroxidation was observed in all the diabetic patients compared to that in controls. Allard et al., [37] have found increased TBARS levels after the treatment with ω-3 fatty acids for 6 weeks, while a few others [38, 39] could not find any significant difference in TBARS levels in their studies. In this study a significant decrease was observed in TBARS levels after the antidiabetic treatment and a further decrease in TBARS levels was observed in diabetic patients after 2M of combined treatment with omega-3 fatty acids and antidiabetic agents. This decrease in lipid peroxides is not associated with glycemic control as there was no significant decrease in the HbA1 levels after the combined therapy. But in the other group of diabetic patients who were treated with oral antidiabetic drugs alone, the decrease in lipid peroxides after three months of therapy was associated with a significant decrease in their HbA1 levels.

The status of antioxidant enzyme activities in diabetic patients is controversial. A few authors [35, 40] have shown increased erythrocyte CAT activity in diabetics, while several others [41, 42] could not detect any change in CAT activity. In this study, CAT activity at baseline was increased in diabetic patients com-
pared to that in controls. The increase in CAT activity in diabetic patients may be a compensatory mechanism against the increased free radical induced peroxidative damage. Not much significant change was observed in CAT activity in the patients who were on combined therapy. Though the mean value of CAT activity after the combined therapy appears lower than that at the baseline the difference between them is not statistically significant (p = 0.087) by paired Student’s t-test.

There are reports of increased [10] decreased [35, 36, 43] and no change [9, 41] in GPx activity in diabetic patients. But in this study the baseline GPx activity was significantly decreased in diabetic patients compared to that in controls. Not much significant change in GPx activity was observed in diabetic patients who were only on antidiabetic treatment, but a significant increase in GPx activity was observed in diabetic patients after the combined treatment. Lemaitre et al., [44] also have observed increased GPx activity in human platelets after the treatment with ω-3 fatty acids and they have explained that the increase of GPx activity in platelets in their study was due to the increased synthesis of GPx.

The reports about the SOD activity in diabetes are controversial, with some authors reporting no change in SOD activity [35, 41] while others reporting increased activity [43]. There are also reports of decreased SOD activity in diabetic patients [45]. In the present study the SOD activity in diabetic patients was comparable with that in controls. In all the diabetic patients antidiabetic treatment or the combined therapy did not show any effect on erythrocyte SOD activity.

The increased GPx activity observed in diabetic patients after the treatment in this study is an interesting finding, as this enzyme is involved in scavenging of the free radical hydrogen peroxide, resulting in decreased free radical induced lipid peroxidation. The decrease in TBARS observed in ω-3 fatty acids treated diabetic patients could be due to the increased activity of GPx, as GPx scavenges lipid hydroperoxides also.

Despite the reported beneficial effects of ω-3 fatty acids in the treatment of cardiovascular diseases, there is a concern that increased intake of ω-3 fatty acids may lead to increased lipid peroxidation. Vericel et al., [48] have reported decreased production of MDA in the platelets of elderly people treated with 150 mg of DHA and 30 mg of EPA per day. Mori et al., [49] have shown that inclusion of 3.6 g of ω-3 fatty acids per day in the low fat diets reduced in vivo lipid peroxidation in dyslipidemic NIDDM patients. In the present study, the decrease in lipid peroxides in diabetic patients was obtained with a dose of 1.8 g of ω-3 fatty acids per day. The decreased lipid peroxidation and increased GPx activity in the diabetic patients treated with the combined therapy could be due to ω-3 fatty acids only as there was no improvement in glycaemic control after the therapy. Whereas in the diabetic patients, who were treated with antidiabetic drugs alone, the decrease in lipid peroxidation was associated with improvement in glycaemic control, without any change in the antioxidant enzyme activities.

In this study other important findings are reduced levels of serum TG and increased levels of HDL-C in diabetic patients who were on combined treatment. From these findings it can be concluded that supplementation of ω-3 fatty acids along with antidiabetic treatment is very effective in reducing the oxidative stress through an improvement in antioxidant enzyme activities, which may contribute in the prevention of vascular complications of diabetes.

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REFERENCES