Serum Lipids & Lipid Mediators in Childhood Nephrotic Syndrome: 
Part II: Effect of Fish Oil, Vitamin E and Garlic Supplementation on 
the Hyperlipidemia and Lipoprotein Abnormalities in Non-Minimal 
Change Disease

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Abstract:

To determine the effects of fish oil, vitamin E and garlic supplementation (single and in 
combinations) on hyperlipidemia and lipoprotein abnormalities resulting from relapsing nephrotic 
syndrome (relapsing NS), 21 children with relapsing NS in remission, with biopsy-proven non-minimal 
change disease (non-MCD), and persistent hyperlipidemia, as well as 10 apparently healthy controls of 
matchable age and sex were included in the study. All the cases and controls were subjected to the 
following investigations: estimation of serum levels of total cholesterol (TC), triglycerides (TG), high 
density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), apolipoprotein A 
(Apo-A), apolipoprotein B (Apo-B), lipid peroxides (LPER), and oxidized low density lipoprotein (Ox-LDL). 
Also, the atherosclerotic index (LDL-C/HDL-C) was calculated. The studied cases were randomly 
assigned for 12 weeks to one of the following nutritional supplements: (1) 6 gm fish oil / d, providing 1.8 
gm n-3 fatty acids; (2) 100 mg vitamin E / d; and (3) 600 mg garlic / d. Then, all the studied parameters 
were reevaluated and all the nutritional supplements were stopped for 8 weeks during which one case 
relapsed and was excluded from the study. At the end of the 8 weeks, the remainder 20 cases were 
randomly assigned for another 12 weeks to one of the following combined nutritional supplements (1) 
fish oil + vitamin E; (2) fish oil + garlic; (3) vitamin E + garlic; and (4) fish oil + vitamin E + garlic. The 
supplements were given in the above-mentioned doses. At the end of the 12 weeks a second reevaluation 
of all the studied parameters was done. Effects of fish oil, vitamin E, and garlic (single or combined) on 
serum lipid fractions are detailed.

The study concluded that, use of single supplement for treatment of hyperlipidemia in NS is 
inadequate to correct all the abnormalities in lipid fractions. Supplementation with either vitamin E or 
garlic led to significant reductions in TC and LDL-C serum levels without any effect on TG serum levels, 
whereas fish oil supplementation normalized TG and HDL-C, but increased LDL-C serum levels 
significantly. Also, the use of two combined supplements was insufficient to normalize all lipid fractions, 
while the combination of the three supplements prevented the fish oil induced rise in LDL-C by the 
synergistic effect of garlic and vitamin E which normalized LDL-C serum levels. Whilst co-administration 
of fish oil, vitamin E and garlic was well-tolerated in the short term and had a beneficial effect on serum 
lipid fractions by providing a combined lowering of TC, LDL-C and TG serum levels along with overall 
reduction in the atherosclerotic index (LDL-C/HDL-C), further controlled studies are required to confirm 
their benefits in children with non-MCD. Such studies need to be large, prospective, and randomized with 
long-term follow up.

Introduction:

Hyperlipidemia and lipoprotein abnormalities are characteristic features of 
nephrotic syndrome (NS)⁴,⁵,⁹. These abnormalities invariably resolve in patients 
undergoing spontaneous or steroid-induced remissions, but persist in resistant forms of NS⁶. 
Lipoprotein abnormalities stimulate the production of lipid mediators including eicosanoids, platelet 
activating factors and chemotactic factors⁴. These biochemical abnormalities could have two major 
adverse consequences: it could contribute to the increased risk of coronary heart disease⁷, and it 
could hasten the progression of renal failure⁸. Interventions to modify lipid abnormalities may 
prevent or postpone these complications. Hence, there has been much interest in the use of lipid 
lowering therapy in hyperlipidemic patients with proteinuric glomerular disease⁹. In nephrotic 
syndrome, several investigators have evaluated the effect of dietary manipulation⁹,¹⁰,¹¹, nutritional 
supplements¹²,¹³,¹⁴,¹⁵ and lipid lowering drugs¹⁶,¹⁷,¹⁸.
on lipid abnormalities. As regards the nutritional supplements, several studies proved beneficial effects of fish oil\(^\text{[12,13,14]}\), garlic\(^\text{[13,19,20]}\) and vitamin E\(^\text{[15,17]}\) on lipid abnormalities in NS.

The purpose of the present study was to determine the effects of single and combined regimens of fish oil, garlic and vitamin E on lipid abnormalities in children with non-MCD.

**Subjects and Methods:**

The study included 21 children with a history of relapsing NS and biopsy-proven non-MCD, aged from 5-13 years (13 boys and 8 girls) as well as 10 apparently healthy children of matchable age and sex as controls. Cases were admitted to the Pediatric Department, Assiut University Hospital. The study was conducted during the period from June 1997 to April 1998.

The studied cases were in remission, and had normal kidney function as defined by serum creatinine <1 mg/dL and creatinine clearance >80 m/min. Also, all of them had persistent hyperlipidemia with TC and LDL-C levels higher than levels at which lipid lowering therapy is recommended: TC >240 mg/dL and LDL-C >160 mg/dL\(^\text{[21,22]}\). Cases taking lipid altering therapies (dietary lipids and proteins intervention, nutritional supplements, and/or blood pressure lowering drugs) within 8 weeks of the beginning of the study, were excluded from the study, as well as cases with a history suggestive of diabetes mellitus, collagen disease, malignant disease and/or familial hypercholesterolemia. Oral consent was obtained from the parents of the subjects and controls before they were recruited into the study.

Beside detailed history and clinical examination, all cases and controls were subjected to the following investigations: complete blood picture, total plasma proteins and albumin / globulin ratio, blood urea, midstream urine culture and bacterial count and estimation of proteinuria / 24 hours.

To determine the lipid fractions, venous blood samples were taken after a 12-14 hours overnight fasting. The serum was separated and stored at -70°C for analysis, to prevent oxidation and proteolytic degradation of lipoproteins, the serum was supplemented with 5 mM EDTA as well as 10 mM butylated hydroxy toluene. Levels of TC, TG, HDL-C, LDL-C were determined by totally enzymatic kits supplied by “Bio-Merieux, France”, code numbers: 61224, 61671, 61531 and 6153 respectively. Determination of serum levels of Apo-A and Apo-B were done by using immunodiffusion plates supplied by “Boehringer Mannheim, West Germany”. The atherosclerotic index LDL-C/HDL-C was calculated\(^\text{[23]}\). Lipid peroxides in serum were determined as the thiobarbituric acid reactive substances (TBARS) by the method of Satoh\(^\text{[24]}\). To determine the level of Ox-LDL, LDL particles were separated using LDL precipitating and solubilizing reagents supplied in the LDL cholesterol / phospholipids kits produced by “Bio-Merieus, France”, the LDL-thiobarbituric acid reactive substances were determined by the method of Satoh\(^\text{[24]}\) and the protein content of LDL was determined as described by Lowry et al.\(^\text{[25]}\).

**Supplements:**

Fish oil containing n-3 fatty acids (Minapharm, Egypt) was given as two 1 g capsules (each containing 180 mg eicosapentaenoic acid (EPA) and 120 mg docosahexaenoic acid (DHA)) three times daily with meals. Vitamin E capsules (Pharco) were given as one 100 mg capsule daily. Garlic tablets (Sekem lab. for biological products, Egypt) were given as one 200 mg tablet three times daily with meals.

**Study design:**

<table>
<thead>
<tr>
<th>Group A: Fish oil (n=7)</th>
<th>Group 1: Fish oil + Vit E (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B: Vit. E (n=7)</td>
<td>Group 2: Fish oil + Garlic (n=5)</td>
</tr>
<tr>
<td>Group A: Garlic (n=7)</td>
<td>Group 3: Vit E + Garlic (n=5)</td>
</tr>
</tbody>
</table>

One case from group A relapsed

<table>
<thead>
<tr>
<th>0</th>
<th>12</th>
<th>20</th>
<th>32</th>
</tr>
</thead>
</table>

*Schematic representation of the study design*
Estimation of all the studied parameters was done at the beginning of the study (0 time), then the studied cases were classified randomly into 3 groups (A, B and C), each included 7 and received a different single supplement (fish oil, vitamin E or garlic) in the previously mentioned doses for 12 weeks after which, all the studied parameters were reevaluated, and all therapies were stopped for 8 weeks during which one case relapsed and was excluded from the study. At the end of this period. The remainder 20 cases were classified randomly into 4 groups (1-4), each included 5 cases. The first group received fish oil + vitamin E, the second received fish oil + garlic, the third received vitamin E + garlic, and the last received fish oil + vitamin E + garlic. These regimens continued for 12 weeks after which a second reevaluation of all the studied parameters was done.

Data was described using mean ± SD and compared using Student’s t-test. Paired data (before therapy and after therapy data) were compared using paired t-test.

Results & Discussion:

Duration of the relapsing NS in the studied cases ranged from 4-9 years (mean duration of illness= 6.699±1.963 ys). In fish oil supplemented group, the atherosclerotic index decreased by 9.61% after therapy, while serum levels of LPER and Ox-LDL increased by 15.7% and 16.62% respectively. Data are shown in tables I-III.

Cases of the present study showed significantly higher TC, TG, LDL-C serum levels as well as the atherosclerotic index, and significantly lower HDL-C serum levels compared with the controls. Several authors reported that these abnormalities are salient features of NS. Both increased production of Apo-B containing lipoproteins and impaired catabolism have been suggested to contribute to the hyperlipidemia. Previous studies reported that, in resistant forms of the NS, hyperlipidemia is usually persistent and may be severe enough to warrant concern regarding possible complications. Also, Zilleruelo et al. found that, persistence and severity of lipid changes correlated well with the duration of the disease. It is noteworthy that all cases of the present study were suffering from relapsing NS for more than 4 years (mean duration of illness = 6.699 ± 1.963 years).

Significantly higher Apo-B serum levels, and significantly lower Apo-A serum levels were found in the studied cases compared with the controls. It was reported that, Apo-A and Apo-B are the mirrors of HDL-C and LDL-C respectively. Apolipoproteins regulate lipoprotein metabolism and determine the unique roles of these lipoproteins in lipid metabolism. Albers et al. have recommended apolipoprotein measurement instead of, or in association with lipoprotein cholesterol measurement, because the laboratory steps involved in the measurement of lipoprotein cholesterol can be subject to a number of potential errors, giving rise to inaccuracy and imprecision.

The present data showed significant elevations in LPER and Ox-LDL serum levels in the studied cases compared with the controls. This may be due to the significantly higher serum levels of LDL-C in the former group than in the latter. LPER serum levels reflect the serum levels of reactive oxygen metabolites which play an important role in the pathophysiology of glomerular disease as it can degrade glomerular basement membrane and induce glomerular injury, cause modifications in LDL that are chemotactic for circulating monocytes, and stimulate synthesis of prostaglandins, leukotrienes and thromboxanes. Also, Ox-LDL causes reduced macrophage mobility and enhances the development of macrophage derived foam cells which may release a variety of pro-inflammatory and pro-fibrotic mediators.

The role of elevated serum TC and LDL-C as well as reduced serum HDL-C in the development of atherosclerosis is well established. Patients with accompanying elevated serum TG concentrations are at increased risk of developing atherosclerosis and coronary artery disease. Both the Prospective Cardiovascular Munster Study and the Helsinki Heart Study suggested that hypertriglyceridemia is a powerful additional risk factor of coronary artery disease, particularly when excessive TG concentrations coincide with a high ratio of LDL-C to HDL-C. In addition, some studies have shown that TG is independently related to coronary artery disease risk and to the extent of coronary atherosclerosis. Effective and safe treatment to reduce the simultaneous elevation of TC and TG concentrations is limited. The use of nutritional supplements either alone or in combination with a drug has been shown to be effective in lowering TC and TG concentrations in hyperlipidemic subjects.

In agreement with previous studies, fish oil supplemented group in the present study showed significantly lower TG serum levels after...
therapy. Fish oil rich in the n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to effectively reduce elevated TG concentrations. This may be due to an inhibition of hepatic fatty acid synthesis by EPA and DHA and impaired TG synthesis\(^{[13,43]}\). Findings from experimental animals, perfused liver systems, and isolated liver cell preparations consistently support that reduced TG synthesis with fish oil supplementation results from substrate diversion away from TG formation\(^{[44]}\).

Table I: Serum levels of lipid fractions, lipid peroxides and oxidized low density lipoprotein in the studied cases compared with the controls

<table>
<thead>
<tr>
<th>Cases</th>
<th>Lipids</th>
<th>Lipoproteins</th>
<th>Apolipoproteins</th>
<th>Athero-sclerotic index (LDL-C/HDL-C)</th>
<th>LPER (Mmol/L)</th>
<th>Ox-LDL (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n=21)</td>
<td>Mean</td>
<td>313.01</td>
<td>138.49</td>
<td>40.84</td>
<td>220.64</td>
<td>130.71</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>26.96</td>
<td>24.54</td>
<td>6.83</td>
<td>9.14</td>
<td>11.52</td>
</tr>
<tr>
<td>II Controls</td>
<td>Mean</td>
<td>141.53</td>
<td>107.98</td>
<td>51.63</td>
<td>80.59</td>
<td>159.48</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>29.24</td>
<td>15.17</td>
<td>6.19</td>
<td>20.12</td>
<td>22.91</td>
</tr>
</tbody>
</table>

Values of p: I vs II

Table II: Serum levels of lipid fractions, lipid peroxides and oxidized low density lipoprotein in relation to the single nutritional supplement

<table>
<thead>
<tr>
<th>Dietary Supplementation</th>
<th>Lipids</th>
<th>Lipoproteins</th>
<th>Apolipoproteins</th>
<th>Athero-sclerotic index (LDL-C/HDL-C)</th>
<th>LPER (Mmol/L)</th>
<th>Ox-LDL (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish oil (n=7)</td>
<td>I Before therapy</td>
<td>Mean</td>
<td>315.38</td>
<td>141.03</td>
<td>40.56</td>
<td>218.84</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>27.59</td>
<td>9.18</td>
<td>8.08</td>
<td>8.62</td>
<td>16.74</td>
</tr>
<tr>
<td>II After therapy</td>
<td>Mean</td>
<td>35.91</td>
<td>114.19</td>
<td>50.29</td>
<td>233.98</td>
<td>147.11</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>21.97</td>
<td>6.03</td>
<td>5.18</td>
<td>10.57</td>
<td>10.28</td>
</tr>
<tr>
<td>Vitamin E (n=7)</td>
<td>III Before therapy</td>
<td>Mean</td>
<td>309.95</td>
<td>139.16</td>
<td>39.82</td>
<td>222.23</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>29.98</td>
<td>11.63</td>
<td>7.91</td>
<td>11.43</td>
<td>18.96</td>
</tr>
<tr>
<td>IV After therapy</td>
<td>Mean</td>
<td>273.42</td>
<td>132.98</td>
<td>41.01</td>
<td>168.91</td>
<td>128.95</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>18.37</td>
<td>10.31</td>
<td>5.32</td>
<td>7.87</td>
<td>12.71</td>
</tr>
<tr>
<td>Garlic (n=7)</td>
<td>V Before therapy</td>
<td>Mean</td>
<td>313.7</td>
<td>135.28</td>
<td>42.13</td>
<td>220.85</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>19.24</td>
<td>9.19</td>
<td>6.21</td>
<td>8.66</td>
<td>17.97</td>
</tr>
<tr>
<td>VI After therapy</td>
<td>Mean</td>
<td>252.99</td>
<td>131.9</td>
<td>40.42</td>
<td>152.46</td>
<td>127.99</td>
</tr>
<tr>
<td></td>
<td>± S.D.</td>
<td>19.01</td>
<td>10.04</td>
<td>5.16</td>
<td>6.39</td>
<td>14.69</td>
</tr>
<tr>
<td>Controls</td>
<td>Mean</td>
<td>141.53</td>
<td>107.98</td>
<td>51.63</td>
<td>80.59</td>
<td>159.48</td>
</tr>
<tr>
<td></td>
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<td>29.24</td>
<td>15.17</td>
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<td>22.91</td>
</tr>
</tbody>
</table>

Values of p:

Values of p: I vs II

NS= Non Significant

All the studied parameters showed no significant differences between the three studied groups before therapy, this confirms matchability of groups in all studied parameters.
Several studies support this hypothesis. Linga et al. noted a reduction in the binding affinity for the LDL receptor. Fish oil supplementation produced two major changes in LDL-C serum levels after fish oil supplementation. They added that, fish oil supplementation on LDL kinetic behavior was to decrease LDL clearance, therefore, LDL apo B uptake suggesting a decrease in receptor mediated LDL uptake. This agrees with several studies showing reduced binding, internalization, and degradation of LDL obtained from fish oil-fed monkeys compared with lard-fed monkeys, when incubated with cultured skin fibroblasts in vitro. Several compositional alterations in LDL were noted, including a reduction in both Apo-E and cholesterol ester content. In subsequent studies, binding to fibroblasts in fish oil-fed monkeys was reduced to a greater extent by monoclonal antibodies to Apo-B than to Apo-E, suggesting that the reduced binding in vitro was mediated by the reduction in Apo-E concentrations. In miniature pigs, fish oil-supplemented animals, compared with corn oil-supplemented controls showed a 17% reduction in native compared with methylated LDL, suggesting a decrease in receptor mediated LDL uptake. On the other hand, Adler et al. reported that...
explained the significant increase in LDL-C concentrations after fish oil supplementation by an increased conversion of very-low density lipoprotein (VLDL) to LDL particles.

In the present study, fish oil supplemented group showed significantly higher serum levels of HDL-C after therapy than before. This was previously reported and explained by increased synthesis. This concomitant increase in serum HDL-C could be beneficial “per se” to reduce the risk of atherosclerosis. It is noteworthy that, in the present study, the atherosclerotic index decreased by 9.61% after fish oil supplementation.

The present data showed that, LPER and Ox-LDL serum levels increased by 15.7% and 16.62% respectively after fish oil supplementation. This may be due to the significant increase in LDL-C serum levels. Increased serum levels of LDL-C, LPER and Ox-LDL after fish oil supplementation is an unwanted effect and is considered a drawback of its use although it normalized TG and HDL-C serum levels.

In the present study, vitamin E supplemented group showed significantly lower serum levels of TC, LDL-C, Apo-B, LPER and Ox-LDL as well as the atherosclerotic index after therapy than before. However, these levels were still significantly higher in comparison with the controls except for LPER and Apo-B. This agrees with previous experimental studies, which concluded that vit. E can improve hyperlipidemia and ameliorate glomerulosclerosis. Studies in experimental NS have incriminated reactive oxygen metabolites in the pathogenesis of the disease and it was found that malondialdehyde (an index of lipoperoxidation) is high in kidney and plasma membranes where it blocks the transfer of electrons involved in the initiation and propagation of the peroxidation of lipids. Vitamin E is important in the protection of lipids from oxidation. It is a liposoluble substance that is present in the lipidic bilayer of the intracellular and plasma membranes where it blocks the transfer of electrons involved in the initiation and propagation of the peroxidation of lipids. Yet, vitamin E supplementation in NS is beneficial.

The present data showed significant reductions in TC, LDL-C and Apo-B serum levels as well as the atherosclerotic index in garlic supplemented group after therapy than before. Silagy and Neil reported that garlic supplementation reduced TC by 9-12% without a significant effect on HDL-C concentrations, and moderately reduced TG concentrations. In a more recent study, garlic supplemented group showed significant reductions in TC and LDL-C serum levels as well as atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C). The decreased serum levels of TC is believed to be largely due to a reduction in LDL-C, which may be due to an inhibition of hepatic cholesterol biosynthesis (possibly via inhibition of hydroxymethylglutaryl-CoA reductase) by allicin and/or other components. Garlic supplemented group in the present study showed also significant reductions in LPER and Ox-LDL serum levels after therapy. This may be due to the reduction in LDL-C serum levels. It is noteworthy that nearly all the above-mentioned parameters which decreased with garlic therapy remained significantly higher compared with the controls.

When we used fish oil, vitamin E and garlic in different combinations, the group that received the three supplements showed the best profile of lipid fractions as well as the studied lipid mediators (LPER and Ox-LDL). This may be due to the synergistic and complementary effects of the three supplements on lipid fractions abnormalities.

In conclusion, use of single supplement for treatment of hyperlipidemia in NS is inadequate to correct all the abnormalities in lipid fractions. Supplementation with either vitamin E or garlic led to significant reductions in TC and LDL-C serum levels without any effect on TG serum levels, whereas fish oil supplementation normalized TG and HDL-C, but increased LDL-C serum levels significantly. Also, the use of two combined supplements was insufficient to normalize all lipid fractions, while the combination of the three supplements prevented the fish oil induced rise in LDL-C by the synergistic effect of garlic and vitamin E which normalized LDL-C serum levels.

Whilst co-administration of fish oil, vitamin E and garlic was well-tolerated in the short term and had a beneficial effect on serum lipid fractions by providing a combined lowering of TC, LDL-C and TG serum levels along with overall reduction in the atherosclerotic index (LDL-C / HDL-C), further controlled studies are required to confirm their benefits in children with non-MCD. Such studies need to be large, prospective, and randomized with long-term follow-up.
References:


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