Serum Lipids & Lipid Mediators in Childhood Nephrotic Syndrome: 
Part I: Effect of Dietary Lipids and Proteins on Serum Lipids and Lipid Mediators in Nephrotic Children
Ekram A. Hashem, Madeha M. Zakhary¹, Enas A. Daef², Sanaa M. Sotohi³ and Mohammad Y. El-Kabsh⁴

From the Departments of Pediatrics, Biochemistry¹, Microbiology and Immunology², Pathology³, and Clinical Pathology⁴, Faculty of Medicine, Assiut University, Egypt.

Abstract:
To study the profile of serum lipids and lipid mediators in nephrotic children, and to investigate the influence of dietary lipids and proteins on this profile, this study was carried out on 32 children who presented with nephrotic syndrome (NS), as well as 10 apparently healthy controls of matchable age and sex. All the patients 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), lipoprotein (a) [LP(a)], apolipoprotein-A (Apo-A), and apolipoprotein B (Apo-B). The atherosclerotic index (LDL-C/HDL-C) was calculated. Also, serum levels of the following lipid mediators were estimated: lipid peroxides (LPER), oxidized low density lipoprotein (Ox-LDL), platelet activating factors (PAF) including platelet factor 4 (PF4), and beta thromboglobulin (BTG), cytokines including glomerular transforming growth factor-beta 1 (TGF-B1), interleukin-1-beta (IL-1-B), and tumor necrosis factor alpha (TNF-α), in addition to vasoactive substances including endothelin-1 (ET-1) and nitric oxide (NO). Percutaneous renal biopsy was done for all steroid dependent and steroid resistant cases. Biopsies were examined by light microscopy and electron microscopy. All the studied parameters were reevaluated during remission, 12 weeks after stopping therapy, then cases with non-minimal change disease (non-MCD) were divided into 4 groups, each fed with a different dietary regimen as regards the intake of fats and proteins. These regimes continued for 12 weeks and followed by a second reevaluation of all the studied parameters.

Alterations in serum lipids and lipid mediators, relation between these alterations and severity of the disease, and influence of dietary fats and proteins on these parameters are detailed.

We concluded that hyperlipidemia in NS may be related to the progression of glomerulosclerosis through a vast array of mediators affecting inflammation, glomerular blood flow and fibrosis. Although dietary intervention appeared to be inadequate to correct all the abnormalities in serum lipids and mediators, it should be the first-line treatment in such cases, since it can be used for long periods of time and is devoid of side effects.

Introduction:
Hyperlipidemia and lipoprotein abnormalities are salient features of nephrotic syndrome⁴. Lipoprotein perturbations stimulate the production of some mediators including eicosanoids, platelet activating factors, and chemotactic factors⁴. Lipoprotein abnormalities and release of lipid mediators accelerate vascular injury leading to atherosclerosis and thromboembolic complications⁵ which increase the risk for cardiovascular morbidity⁶. Also, these abnormalities contribute to the process of glomerulosclerosis and progression to end-stage renal disease ⁴. Previous studies proved some influence of dietary lipids⁹ and proteins¹⁰ on lipid abnormalities in nephrotic syndrome.

The present study aimed to investigate the profile of serum lipid fractions and lipid mediators in nephrotic children, to detect the relation between this profile and severity of the disease, and to investigate whether dietary intervention will influence this profile.

Subjects and Methods:
Thirty-two patients aged from 4-12 years (20 boys and 12 girls) with nephrotic syndrome, diagnosed according to International Study of Kidney Disease in Children¹², were included in this study, in addition to 10 apparently healthy children of matchable age and sex as controls. Patients were admitted to the Pediatric Department, Assiut University Hospital. The study was conducted...
All patients had normal kidney function as defined by serum creatinine <1 mg/dL and creatinine clearance >80 ml/min. Patients with a history suggestive of diabetes mellitus, hypothyroidism, collagen disease, malignant disease and/or familial hypercholesterolemia were excluded from the study. Oral consent was obtained from the parents of the subjects and controls before they were recruited into the study.

Beside meticulous history and thorough clinical examination, all patients and controls were subjected to the following investigations: complete blood picture, total plasma proteins and albumin/globulin ratio, midstream urine culture and bacterial count and estimation of proteinuria / 24 hours, as well as studying the profile of lipid fractions and lipid mediators.

To study the profile of lipid fractions and lipid mediators, 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 according to Prata et al.[13]. Lipoprotein (a) was determined by an enzyme immunoassay (ELISA) kit supplied by Innogenetics, Belgium, Catalog number K-1015/950207. Lipid peroxides in serum were determined as thiobarbituric acid reactive substances (TBARS) by the method of Satoh[14]. 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-Merieux, France", the LDL-thiobarbituric acid reactive substances were determined by the method of Satoh[14], and the protein content of LDL was determined as described by Lowry et al.[15]. TGF-B1 was determined using a quantitative sandwich enzyme immunoassay kit: Quantikine human TGF-B1 supplied by R & D systems, Inc, Minneapolis, USA. The minimum detectable dose of TNF-α was determined to be 7.5 pg/ml. IL-1-B was assayed by ELISA technique using Quantikine human IL-1-B immuno-assay kit, supplied by R & D systems, Inc., Minneapolis, USA. The minimum detectable dose of IL-1-B was 0.3 pg/ml. ET1 was measured by competitive enzyme immunoassay (EIA) using a kit, supplied by Peninsula Laboratories. NO decomposes in vivo to nitrite plus nitrate. Their levels in blood were measured as an index of reactive nitrogen intermediates. The nitrate was reduced to nitrite by incubation with cadmium filings, the total concentration of nitrite was then measured using the Griess reaction[16]. PF4 was determined using sandwich enzyme immunoassay kit supplied by Stago diagnostic Ltd. Company, Leon, France. BTG was determined by ELISA technique using kits supplied by Behring werg AG, Germany.

Percutaneous renal biopsy was done for all steroid dependent and steroid resistant cases. Biopsies were examined by light microscopy and electron microscopy.

These parameters were reevaluated during remission, 12 weeks after stopping all therapies. Patients who were proved to have non-MCD by renal biopsy (21) were divided into 4 groups, each group fed with a different dietary regimen as regards the protein and fat intake. Five fed with high protein / normal fat diet, 5 fed with high protein / restricted fat diet, 5 fed with normal protein / normal fat diet and the last 6 patients fed with normal protein / restricted fat diet. These regimens continued for 12 weeks after which a second reevaluation of all the studied parameters was done. The approximate protein content in food was calculated according to Alpers et al.[17]. High protein diet contained 3-4 gm/kg body weight / d[18], and normal protein diet contained 1-2 gm/kg body weight / d[18]. Also, we prescribed the restricted fat diet according to the guidelines mentioned by Alpers et al.[19].

Data was described using mean ± SD and compared using Student's t-test. Paired data (at admission and during remission data) were compared using paired t-test.

**Results:**

Among the 32 studied cases, 10 were steroid responsive, 14 were steroid dependent and 8 were steroid resistant. Renal biopsy of the 22 steroid dependent and steroid resistant cases revealed the following diagnoses: minimal change...
nephropathy in one patient, focal segmental glomerulosclerosis (FSGS) in 9 patients, mesangiocapillary glomerulonephritis in 7 patients, mesangial proliferative glomerulonephritis in 3 patients and membranoproliferative glomerulonephritis (MPGN) in 2 patients. Patients with minimal change disease (MCD) were enrolled in the study with the first attack of the disease while all patients with non-MCD were suffering from the disease for ≥ 3 years (range 3-8 years, and mean duration of illness = 5.619 ± 1.963 years).

After the normal protein / normal fat regimen, TC and LDL-C decreased by 3.17% and 1.82% respectively. After the high protein / restricted fat regimen, TC and LDL-C decreased by 15% and 8.3% respectively, and after the normal protein / restricted fat regimen, LDL-C and the atherosclerotic index decreased by 12.18% and 9.97% respectively and HDL-C increased by 12.78%.

Data are shown in tables I-IV and figures 1 and 2.

### Table I: Serum levels of lipid fractions and atherosclerotic index in nephrotic children compared with the controls

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>TG</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>Lp(a)</th>
<th>Apo-A</th>
<th>Apo-B</th>
<th>Atherosclerotic index LDL-C / HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I Controls</strong></td>
<td><strong>Mean</strong></td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
</tr>
<tr>
<td>n(10)</td>
<td>141.53</td>
<td>29.24</td>
<td>107.98</td>
<td>6.19</td>
<td>51.63</td>
<td>19.12</td>
<td>5.39</td>
<td>2.336</td>
</tr>
<tr>
<td><strong>II Patients at admission</strong></td>
<td><strong>Mean</strong></td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
</tr>
<tr>
<td>n(32)</td>
<td>361.136</td>
<td>29.26</td>
<td>136.037</td>
<td>6.31</td>
<td>44.295</td>
<td>19.97</td>
<td>46.38</td>
<td>5.864</td>
</tr>
<tr>
<td><strong>Value of p : II vs I</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table II: Serum levels of lipid fractions and atherosclerotic index in relation to the histopathology

<table>
<thead>
<tr>
<th></th>
<th>Lipids (mg/dL)</th>
<th>Lipoproteins (mg/dL)</th>
<th>Apolipoproteins (mg/dL)</th>
<th>Atherosclerotic index LDL-C / HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with MCD</strong></td>
<td><strong>Mean</strong></td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
</tr>
<tr>
<td>I At admission</td>
<td>237.36</td>
<td>52.91</td>
<td>111.25</td>
<td>3.12</td>
</tr>
<tr>
<td>II During remission</td>
<td>158.28</td>
<td>21.39</td>
<td>106.96</td>
<td>4.28</td>
</tr>
<tr>
<td><strong>Patients with non-MCD</strong></td>
<td><strong>Mean</strong></td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
</tr>
<tr>
<td>III At admission</td>
<td>425.97</td>
<td>16.87</td>
<td>149.02</td>
<td>6.54</td>
</tr>
<tr>
<td>IV During remission</td>
<td>353.94</td>
<td>9.42</td>
<td>124.39</td>
<td>8.38</td>
</tr>
<tr>
<td><strong>V Controls</strong></td>
<td><strong>Mean</strong></td>
<td>± S.D.</td>
<td>± S.D.</td>
<td>± S.D.</td>
</tr>
<tr>
<td>n(10)</td>
<td>141.53</td>
<td>29.24</td>
<td>107.98</td>
<td>6.19</td>
</tr>
<tr>
<td><strong>Value of p :</strong></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**NS = non significant**
Table III: Serum levels of lipid mediators in nephrotic children compared with the controls

<table>
<thead>
<tr>
<th></th>
<th>LPER (nmol/L)</th>
<th>Ox-LDL (mg/dL)</th>
<th>PAF (%)</th>
<th>BTG (%)</th>
<th>TGF-B1 (pg/ml)</th>
<th>IL-1B (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>ET-1 (ng/ml)</th>
<th>NO (nmol/mg ptn.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Controls (n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>II Patients at admission (n=32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Significance of differences: II vs I</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.06</td>
</tr>
</tbody>
</table>

Table IV: Serum levels of lipid mediators in relation to the histopathology

<table>
<thead>
<tr>
<th>Patients with MCD (n=11)</th>
<th>LPER (nmol/L)</th>
<th>Ox-LDL (mg/dL)</th>
<th>PAF (%)</th>
<th>BTG (%)</th>
<th>TGF-B1 (pg/ml)</th>
<th>IL-1B (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>ET-1 (ng/ml)</th>
<th>NO (nmol/mg ptn.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I At admission</td>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>II During remission</td>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Patients with non-MCD (n=21)</td>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>I At admission</td>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>IV During remission</td>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>V Controls (n=10)</td>
<td>Mean</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>S.D.</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Value of p</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>I vs III</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.005</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>I vs II</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>III vs IV</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>II vs V</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IV vs V</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS= Non significant

Discussion:

Nephrotic children in the present study showed significantly higher serum levels of TC, LDL-C and TG compared with the controls. Several authors reported that these abnormalities are well-established features of the NS. Both increased production of Apo-B-containing lipoproteins (VLDL + LDL) and impaired catabolism have been suggested to contribute to the hyperlipidemia. It is assumed that hepatic lipoprotein synthesis is stimulated in response to hypoalbuminemia, low oncotic pressure, and urinary albumin loss. Decreased renal clearance of mevalonate provided a substrate for hepatic lipogenesis. On the other hand, decreased catabolism of LDL-C by the receptor-mediated pathway may also be involved in the pathogenesis of nephrotic hyperlipidemia. Increased intracellular cholesterol may reduce the number of LDL receptors on the liver cell surface. Uptake of LDL-C by the liver, the major pathway of LDL-C removal from the plasma, may thereby be decreased.
Fig. (1): Serum levels of lipid fractions and atherosclerotic index in relation to the dietary regimen.

- **HP/NF**: High protein / normal fat
- **HP/RF**: High protein / restricted fat
- **NP/NF**: Normal protein / normal fat
- **NP/RF**: Normal protein / restricted fat

All the studied parameters showed no significant differences between the four studied groups before therapy, this confirms matchability of groups in all studied parameters.

* = p<0.05 compared with before therapy
** = p<0.025 compared with before therapy
Fig. (2): Serum levels of lipid mediators in relation to the dietary regimen

- **LPER**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **Ox-LDL**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **PF4**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **BTG**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **IL-1**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **TGF-B1**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **NO**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **ET1**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

- **TNF**
  - Control (n=10)
  - HP/NF (n=5)
  - HP/RF (n=5)
  - NP/NF (n=5)
  - NP/RF (n=6)

**Legend:**
- Control
- Before therapy
- After therapy

**Groups:**
- HP/NF: High protein / normal fat
- HP/RF: High protein / restricted fat
- NP/NF: Normal protein / normal fat
- NP/RF: Normal protein / restricted fat

All the studied parameters showed no significant differences between the four studied groups before therapy, this confirms matchability of groups in all studied parameters.

- * = p<0.05 compared with before therapy
- ** = p<0.02 compared with before therapy
- *** = p<0.01 compared with before therapy
- **** = p<0.005 compared with before therapy

Alex J Pediatr, 12 (2), July 1998
Cases of the present study showed significantly lower serum levels of HDL-C compared with the controls. It was reported that in NS, HDL-C may be either decreased, unchanged or increased\(^{(2)}\). In a study by Nayak et al.,\(^{(29)}\) they found decreased serum levels of HDL-C in nephrotic children. They suggested that this may be due to increased fractional catabolic rate of HDL due to the deficiency of lecithin cholesterol acyl transferase (LCAT) activity, and abnormally high excretion of HDL through damaged glomerular filter. Increased LDL-C and decreased HDL-C are strong risk factors for accelerated atherosclerosis\(^{(8)}\). It is noteworthy that the atherosclerotic index LDL-C/HDL-C was significantly higher in cases of the present study compared with the controls.

Cases of the present study showed significantly higher serum levels of Lp(a) compared with the controls. This is in agreement with several studies\(^{(27,28,29)}\). The high Lp(a) levels suggest an upregulation of the hepatic synthesis of Apo (a) more likely than a decrease in its catabolism\(^{(27)}\). Lipoprotein (a) interferes with the fibrinolytic process\(^{(30)}\) and promotes formation of foam cell \(^{(31)}\), which may release a variety of pro-inflammatory and pro-fibrotic mediators\(^{(4)}\). Therefore, it may contribute to the microthrombosis and lipid deposition in the kidney seen in NS\(^{(29,32)}\).

Apolipoproteins of the various lipoproteins regulate lipoprotein metabolism and determine the unique roles of these lipoproteins in lipid metabolism\(^{(33)}\). Albers et al.\(^{(34)}\) 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. Buser et al.\(^{(35)}\) reported that Apo-A and Apo-B are the mirrors of HDL-C and LDL-C respectively. In agreement with this report, in the present study, nephrotic children showed significantly higher serum levels of Apo-B and significantly lower serum levels of Apo-A in comparison with the controls.

In the present study, all the aforementioned lipid abnormalities were significantly more marked in nephrotic children with non-MCD than in those with MCD. Wiecek et al.\(^{(36)}\) reported that the percentage of damaged glomeruli is the main factor influencing the magnitude of abnormal serum lipid profile in nephrotic patients, while Wheeler and Bernard\(^{(3)}\) reported that, increased serum lipids may contribute to renal injury by: glomerular and tubulo-interstitial lipoprotein deposition, LDL oxidation, mononuclear cell infiltration, lipoprotein-induced cytocytotoxicity and increased matrix synthesis. The hyperlipidemic state may result in increased production of pro-inflammatory lipids and pro-fibrotic mediators, including reactive oxygen species, vasoactive compounds, mitogens, pro-coagulative factors, complement components, proteases\(^{(6)}\), and cytokines\(^{(1,4)}\).

As regards the lipid mediators, cases of the present study showed significantly higher serum levels of LPER and Ox-LDL compared with the controls. Raised serum levels of LPER in nephrotic children indicates increased serum levels of reactive oxygen metabolites\(^{(37)}\). Several in vitro and in vivo studies\(^{(3,4,38)}\) indicate an important role of reactive oxygen metabolites in the pathophysiology of glomerular disease. It can degrade glomerular basement membrane and induce glomerular injury\(^{(3,39)}\), cause modifications in LDL that are chemotactic for circulating monocytes\(^{(3)}\), and stimulate synthesis of prostaglandins\(^{(4,38)}\), leukotrienes (B\(_4\), C\(_4\) and D\(_4\)) and thromboxanes\(^{(4)}\). Leukotriene B\(_4\) is chemotactic for neutrophils and may also affect glomerular filtration, arteriolar resistance, and mesangial contractility. Leukotrienes C\(_4\) and D\(_4\) are highly mitogenic for glomerular epithelial cells\(^{(4)}\). Thromboxane A\(_2\) (TxA\(_2\)) mediates renal vasoconstriction, decreases the glomerular ultrafiltration coefficient and may itself induce proteinuria\(^{(39)}\).

Increased Ox-LDL in NS has been reported by several authors\(^{(3,4,40)}\). Oxidized 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, including reactive oxygen metabolites\(^{(4,38)}\), leukotrienes, thromboxanes, PAF, cytokines (IL-1-B, TNF-α and TGF-B)\(^{(4)}\), and ET1\(^{(40,41)}\).

The present data showed significant elevations in PF4 and BTG serum levels in the studied cases compared with the controls. Increased synthesis of PAF has been demonstrated in nephrotic serum in previous studies\(^{(42,43)}\). Actions of PAF include stimulation of platelet aggregation and activation, chemotaxis and chemokinesis, oxygen free radical formation, and increased vascular permeability\(^{(42)}\); all of these actions could be instrumental in the progression of glomerulosclerosis\(^{(4)}\).
Cases of the present study showed significantly higher serum levels of IL-1-B and TNF-α compared with the controls. This may be due to enhanced production\(^{(4)}\). Previous studies revealed that, Ox-LDL stimulates release of these cytokines\(^{(44,45)}\). IL-1 and TNF are usually synthesized and secreted simultaneously. TNF-α is pleiotropic in its activities and has even more toxic side effects in humans than IL-1. It enhances procoagulant activity in endothelial cells, stimulates fibroblast growth, and stimulates the production of granulocyte-macrophage colony stimulating factor, PAF and IL-1\(^{(46)}\). Both IL-1 and TNF-α cause renal toxicity. But, TNF-α tends to produce more capillary leakage and protein turnover than IL-1\(^{(1,46,47)}\).

The present data showed a significant elevation in TGF-B1 serum levels in the studied cases compared with the controls. Several studies investigated the relationship between hypercholesterolemia, and TGF-B upregulation in nephrotic syndrome. Results of these studies support the hypothesis that activated macrophage – due to hyperlipidemia and increased Ox-LDL - is the cellular source for increased TGF-B\(^{(48,49)}\). TGF-B is a 25-kD polypeptide member of a family of multifunctional polypeptides that affect proliferation, differentiation and numerous other functions within many cell types. TGF-B\(_2\) specifically has an intense stimulatory effect on the gene expression of many extracellular matrix proteins including the collagens, fibronectin, and tenascin\(^{(48)}\). Therefore, it was hypothesized that enhanced production of TGF-B\(_2\) by infiltrating glomerular macrophages could drive increased production of extra-cellular matrix proteins leading to glomerulosclerosis\(^{(4)}\).

Accordant with the present study, previous studies reported an increase in release of ET\(_1\)\(^{(41,50)}\) and a decrease in release of NO\(^{(46)}\) in cases with hyperlipidemia. Boulanger et al.\(^{(41)}\) reported that, in hyperlipidemia, Ox-LDL may be an endogenous mediator of the augmented release of ET\(_1\). The increased production of ET\(_1\) could contribute to vasospastic events and may promote vascular smooth muscle proliferation and progression of atherosclerotic vascular disease\(^{(41)}\). In addition, ET\(_1\) was found to raise the maximum capacity for lipoprotein uptake in human mesangial cells by about twofold\(^{(51)}\). Furthermore, it was reported that LDL and Ox-LDL inhibit NO vasodilatory responses, an action which when added to the abnormal levels of both ET\(_1\) and NO may result in decreased renal tissue oxygenation\(^{(50)}\). The present data showed that, all the abnormalities in lipid mediators were significantly more marked in cases with non-MCNS than in those with MCNS. This may be due to the significant increase in serum levels of LDL-C in the former group compared with the latter.

In the present study, during remission and after stopping all therapies for 12 weeks, cases with MCD showed complete improvement in all the studied parameters while cases with non-MCNS showed only partial improvement nearly in all the studied parameters (tables 2 and 4). This is in agreement with Ordonez et al.\(^{(53)}\) who reported that hyperlipidemia in NS tends to resolve once spontaneous or steroid-induced remission has been achieved. However, in resistant forms of the NS, hyperlipidemia is usually persistent and may be severe enough to warrant concern regarding possible complications. Therefore, it is quite possible that patients with long-standing NS are at an increased risk of such a complication\(^{(53)}\). In a study by Zilleruelo et al.\(^{(54)}\) they found that, persistence and severity of lipid changes correlated well with duration of the disease. It is noteworthy that, in the present study, all cases with non-MCNS were suffering from the disease for more than 3 years (mean duration of illness = 5.619±1.963 years). Meanwhile, all cases with MCD were enrolled in the study with the first attack of the disease.

Significant hypercholesterolemia was identified as a predictor of progression of childhood MPGN\(^{(4)}\). Maneuvers lowering serum cholesterol are associated with a decline in glomerular macrophage number, while worsening hyperlipidemia correlates with increasing glomerular inflammation\(^{(55,56)}\). On theoretical grounds, there is little doubt that the most physiologic and harmless approach to the problem of correcting hyperlipidemia or preventing its occurrence, which could be an alternative to the use of lipid lowering drugs or complementary to it, is dietary intervention\(^{(11)}\). Several investigators have evaluated the effect of dietary manipulation on the hyperlipidemia in NS and proved some influence of dietary lipids on lipid abnormalities\(^{(2,9,10,11)}\). Determining optimal dietary protein levels in NS is a complicated matter. In the past, high-protein diets (3-4 gm/kg body weight / day)\(^{(29)}\) were often prescribed in NS in an attempt to compensate for urinary protein losses and to restore normal serum albumin levels. Now, however, there is less agreement about the utility of this strategy. Dwyer\(^{(57)}\) reported that increased dietary protein does not
correct low serum albumin levels completely but does increase urinary protein excretion and may accelerate renal damage. Watson and Coleman\(^{(18)}\) reported that, a protein intake of 1-2 gm/kg body weight / day should be adequate for most nephrotic children.

In the present study, we investigated the influence of the dietary intake of lipids and proteins on the abnormalities of lipid fractions and lipid mediators in nephrotic children. Very slight improvement was observed in the group fed with normal protein / normal fat regimen as serum levels of TC and LDL-C decreased by 3.17% and 1.82% respectively. More improvement was observed in the group fed with high protein / restricted fat regimen as serum levels of TC and LDL-C decreased by 15% and 8.3% respectively. This agrees with previous reports\(^{(2,9,10)}\). Olbricht\(^{(2)}\) reported that, low fat diet alone is unlikely to be of much benefit in most patients since the expected 10-15% decrease in serum TC are insufficient with respect to the high levels of cholesterol in NS.

The most improvement in the present study was found in the group fed with normal protein / restricted fat regimen. Serum levels of TC and TG decreased significantly. Also, LDL-C serum level and the atherosclerotic index decreased by 12.18% and 9.97% respectively. Furthermore, HDL-C serum level increased by 12.78%. In addition, significant reductions in the serum levels of LPER, Ox-LDL and TGF-B\(_1\), were found. These findings, to some extent, agrees with D’Amico and Gentile\(^{(11)}\) who found that, low protein / low fat regimen for 6 months corrected hypertriglyceridemia and hypercholesterolemia. In their study, serum TC and LDL-C were reduced 24% and 27% respectively.

From the study we concluded that:

- Hyperlipidemia of the NS may be related to the progression of glomerulosclerosis through an increasingly vast array of lipid mediators affecting inflammation, glomerular blood flow and fibrosis.

- Although dietary management appeared to be inadequate to correct all the abnormalities in lipid fractions and lipid mediators profile, dietary intervention should be the first line treatment for the dyslipidemia of NS, since it can be used for long periods of time and is devoid of side effects so long as good nutritional status is maintained.

- Prospective controlled studies will be needed to evaluate the long-term efficacy of normal protein / restricted fat diet in a larger patient population and also to assess whether reduction in cholesterol decreases the risk for atherosclerosis and inhibits the progression of glomerular disease.

References:


