Note

Effects of the Marine Unicellular Alga *Nannochloropsis* sp. to Reduce the Plasma and Liver Cholesterol Levels in Male Rats Fed on Diets with Cholesterol

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The effects of *Nannochloropsis* were studied on rats consuming hypercholesterolemic diets. The whole biomass and the hexane/ethanol extract increased the plasma and hepatic eicosapentaenoic and docosahexaenoic acids levels, and reduced the cholesterol levels. We also observed a higher level of propionate, and a lower ratio between acetate and propionate. These data suggest the efficacy of *Nannochloropsis* in reducing cholesterol levels.

Key words: *Nannochloropsis* sp.; cholesterol; plasma; liver; rat

Feeding eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of the ω3 family has been suggested to play a role in regulating the levels of blood lipids.1–3) Marine unicellular algae, from several taxonomic groups, produce high amounts of polyunsaturated fatty acids (PUFA) and can be incorporated into the human diet. DHA-enriched oils from algae can be incorporated into infant formulas and formulated into commercial nutraceutical foods for infants and the elderly.4) We have previously shown that the marine unicellular algal, *Nannochloropsis* sp., is a rich source of EPA5) that can efficiently be incorporated into the blood, liver, and brain lipids of rats.5,6) In the present study, we evaluated the effectiveness of dietary *Nannochloropsis* biomass and its lipid fraction to reduce the cholesterol levels in rats fed on high-cholesterol diets.

*Nannochloropsis* was obtained from Natural Beta Technologies Ltd., Eilat, Israel, the cells being disrupted and freeze-dried.5) An algal lipid extract was prepared by using Soxhlet apparatus with consecutive extraction by ethanol and hexane, the residual solvents being evaporated under vacuum.

Male Sprague-Dawley rats (75–80 g, each) were housed in individual cages in a controlled environment (23°C and 12-h light/dark cycle) with free access to food and water. The animals were kept and treated according to the guidelines laid down by the Ethics Committee of Technion for experimentation on animals. Thirty-two rats were randomly divided into four groups, each being fed the standard diet recommended by the American Institute of Nutrition7) supplemented with cholesterol (10 g/kg of diet) and cholic acid (2 g/kg of diet). The rats in the control group only, consumed this high-cholesterol diet, while the diet of the three experimental groups was further supplemented either with whole freeze-dried *Nannochloropsis* (100 g/kg of diet), algal lipid extract (35 g/kg of diet), or the algal residue (65 g/kg of diet) that remained after consecutive extraction with ethanol and hexane. The animals were killed after 30 days by CO2 asphyxiation, blood being collected from the abdominal aorta and separated by centrifugation. Following its perfusion with cold isotonic saline, the liver was removed, blotted, and weighed. The cecum was then excised and weighed, its content being collected, weighed, and quickly immersed in liquid nitrogen. All samples were stored at −80°C until needed for analysis. Plasma cholesterol was analyzed by a commercial kit, and hepatic cholesterol by a colorimetric method8) after saponification. The fatty acid profile of the plasma and liver was determined as previously described,5) and the short chain fatty acid (SCFA) levels in the cecum content were determined according to Mathers and Tagny.9)

The main finding of this study is the significant reduction in plasma and liver cholesterol levels resulted from the diets supplemented either with the whole algal biomass or its lipid extract (Fig. 1). The effect of the diet supplemented with the algal residue was less pronounced, showing only mild reduction of the plasma cholesterol levels, with no effect on the hepatic cholesterol content. Rats fed on either the whole algal biomass or its lipid extract uniquely demonstrated the presence of EPA and a significantly high level of DHA in the hepatic lipids (Table 1). Similar
results were obtained for the plasma lipids (data not shown). The weights of the cecum and its content were significantly lower in the rats fed on the diet containing the lipid extract than those from the other dietary groups. No effect was apparent on the acetic acid level in the cecum content. However, feeding the diets supplemented either with the whole biomass or the lipid extract resulted in a significantly high level of propionic acid, and a lower ratio between acetate and propionate than that in the control group or the group fed on the diet supplemented with the algal residue (Table 1).

These results are the first to demonstrate the cholesterol-lowering properties of *Nannochloropsis* in rats, a nutritional model widely used to study the dietary effects on lipid metabolism. The algal biomass was the most effective in this respect, while the diet supplemented with the algal lipid extract demonstrated a significant but lesser hypocholesterolemic effect. The algal residue remaining after ethanol-hexane extraction showed only a minor cholesterol-lowering effect, although being significant compare to the control. The presence of EPA in the plasma and liver lipids of rats fed the whole algae and its lipid extract is indicative of the bioavailability of algal EPA from the diet, and substantiates our previous findings.5,6 Furthermore, rats fed on these diets exhibited a higher hepatic DHA level, showing the conversion of EPA to DHA.6 It is therefore suggested that algal EPA contributed to the significant hypocholesterolemic effect. However, the role of an additional dietary component is emerging as a relatively high level of propionic acid was found in the cecum of the animals fed with either whole algal biomass or with the lipid extract. The production of propionate by the fermentation of soluble fibers in the cecum has been suggested to be partly responsible for the cholesterol-lowering effect of these fibers due to the inhibition of hepatic cholesterol synthesis by this SCFA.6,10 Indeed, the occurrence of dietary fiber in *Nannochloropsis* has been indicated by Markovits et al.11 It is conceivable that the fermentable algal soluble fibers are also present in the lipid extract, and therefore an enhanced propionate level was observed in rats fed this fraction. Additional hypocholesterolemic components, namely phytosterols,12 were found in whole *Nannochloropsis*, and in the algal lipid-extract. These compounds exert their effect by inhibiting intestinal cholesterol absorption.13 On the other hand, the lower hypocholesterolemic effect observed in rats fed with the algal remains is probably due to the presence of insoluble fibers, which are assumed to be the main fiber component in this fraction. Insoluble fibers are believed to influence plasma cholesterol by their modification of bile acid absorption and metabolism.14 It is conceivable that the unique combination of

### Table 1. Hepatic Eicosapentaenoic and Docosahexaenoic Acid Contents (% of total fatty acids), Cecum Weight, Cecal Content Weight, and Its Short-chain Fatty Acid Composition (% of total fatty acids) of Male Rats Fed on Diets Containing Cholesterol (10 g/kg) and Supplemented with *Nannochloropsis* Biomass for 30 Days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Whole</th>
<th>Lipid extract</th>
<th>Algal residues</th>
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</thead>
<tbody>
<tr>
<td><strong>Hepatic FA:</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EPA (20:5)</td>
<td>ND</td>
<td>6.38±1.60</td>
<td>7.08±1.90</td>
<td>ND</td>
</tr>
<tr>
<td>DHA (22:6)</td>
<td>3.28±1.2⁴</td>
<td>5.35±0.86⁴</td>
<td>5.92±2.32⁴</td>
<td>3.83±0.36⁴</td>
</tr>
<tr>
<td><strong>Cecum, g</strong></td>
<td>1.79±0.23⁴</td>
<td>2.06±0.56⁴</td>
<td>1.19±0.18⁵</td>
<td>2.13±0.53⁵</td>
</tr>
<tr>
<td>Cecum content, g</td>
<td>1.19±0.23⁴</td>
<td>1.45±0.57⁴</td>
<td>0.67±0.19⁵</td>
<td>1.50±0.51⁵</td>
</tr>
<tr>
<td><strong>Cecum SCFA:</strong></td>
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<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>31.32±2.35</td>
<td>29.25±5.06</td>
<td>32.94±10.95</td>
<td>27.54±4.11</td>
</tr>
<tr>
<td>Propionic</td>
<td>29.80±6.49⁵</td>
<td>38.56±4.19⁵</td>
<td>38.16±5.55⁵</td>
<td>25.80±4.95⁵</td>
</tr>
<tr>
<td>Butyric</td>
<td>30.85±7.73⁴</td>
<td>22.61±5.17⁴</td>
<td>25.95±5.34⁴</td>
<td>30.91±9.16⁴</td>
</tr>
<tr>
<td>Acetic/Propionic</td>
<td>1.14±0.35⁴</td>
<td>0.77±0.16⁴</td>
<td>0.74±0.19⁴</td>
<td>1.35±0.61⁴</td>
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</tbody>
</table>

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ND, non-detectable; FA, fatty acids; SCFA, short chain fatty acids. Each value is the mean±SD, n=8 rats. Values within a row without a common superscript letter differ significantly (P<0.05).
soluble and insoluble fibers as well as the EPA and phytosterols present in Nannochloropsis played a crucial role in the hypocholesterolemic effect of this alga.

References


