Investigations Regarding Obesity Induction and Prevention through a Guided Diet

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Abstract. Obesity is associated with a high risk of developing diabetes and cardiovascular diseases. Therefore, management of body weight to prevent obesity remains as an important priority. The present investigation addresses the effects of Omega-3 PUFAs on body weight, blood composition and histological aspects in guinea pigs. We used 21 Guinea pigs, 12 weeks old, which were randomly assigned to control group, 0.5 % cholesterol in diet group and 0.5 % cholesterol in diet plus Omega-3 group. Guinea pigs fed with 0.5 % cholesterol in diet showed a significant increase in body weight and serum levels – total cholesterol (TC), triglycerides (TG) and LDL cholesterol (LDL-C) – when compared to control group (P=0.0039, P=0.0095, P=0.00001, P=0.00134 respectively). They also develop severe hepatic steatosis. Adding Omega-3 in diet significant reduce body weight, serum levels – TC, TG and LDL-C and hepatic steatosis when compared to control group (P=0.04077, P=0.01, P=0.00001, P=0.0995). In conclusion, concerning the efficacy of Omega-3 fatty acids to reduce body weight, hypertriglyceridemia and hepatic steatosis, their adding in diet have positive effects in obesity prevention.

Keywords: obesity, polyunsaturated fatty acids, DHA, EPA, high fat diet, body weight

INTRODUCTION

Many studies in rodents have demonstrated that PUFA, especially n-3 PUFA EPA (20:5n-3) and DHA (22:6n-3), which are abundant in marine fish oils, are less effective in promoting accumulation of adipose tissue than saturated fats (Takahashi and Ide, 1999; Azain, 2004; Raclot et al., 1997.). Dietary n-3 PUFA admixed to high-fat (HF) diets do not affect food consumption (Ikemoto et al., 1996; Hun et al., 1999; Oudart et al., 1997), but they do modulate fuel partitioning by down regulating lipogenesis and stimulating lipid oxidation. However, the mechanism for the reduction of body fat stores is still unclear (Lapillonne et al., 2004). Both in animals (Raclot and Oudart, 1999) and humans (Ruxton et al., 2004; Mori et al., 1999), EPA/DHA lower blood TG and may improve insulin sensitivity. Collectively, these benefits resulted in a decline of excess fat building up in the liver – preventing the very serious metabolic problem of fatty liver known as NASH (nonalcoholic steatohepatitis) (Byron, 2009).

Also studies in mice fed semisynthetic high fat diets have documented the reduction of adiposity by n-3 PUFA (Cha et al., 2001; Tsuboyama-Kasaoka et al., 1999) and indicated that EPA and DHA could be more effective than n-3 PUFA of plant origin, that is, a-linolenic acid (ALA, 18:3 n-3). The goal of this study was to learn whether EPA and DHA could limit the
obesity and proliferation of adipose tissue cells induced in the C57BL/6J mice by the composite high fat diet.

The aim of this work consists in study the possibilities to identify functional food models to prove functional methods in human, using animals experimental techniques, consisting in obesity induction through cholesterol and its prevention using dietary supplements with Omega-3 fatty acids.

MATERIALS AND METHODS

Materials
Concentrated fish oil was extracted from Omacor capsules (Pronova, Norway), cholesterol was obtained from sheep wool powder, ~95% (GC), (Sigma-Aldrich), and the standard chow for Guinea pigs was obtained from Pannonmill Takarmany Kft., Hungary.

Animals and diets
Twenty-one male Guinea pigs, 3 months old were placed on 1 of 3 groups: control group fed with a standard chow, high fat group fed with 0.5% cholesterol in diet and Omega-3 group fed with 0.5% cholesterol plus 0.6 mg/kg Omega-3 PUFA in diet. The animals were housed in cages and maintained in a temperature-controlled room on a 12:12-h dark–light cycle for 8 weeks. The body weight was measured once at two weeks and serum parameters were determined at baseline and after 4 and 8 weeks. At the end of the 8 weeks, animals were sacrificed for histological slides.

Serum determinations
Fasting serum samples were obtained for cholesterol and triglyceride determinations at baseline and after 4 and 8 weeks on the experimental diet. The blood was collected from the retro orbicular sinus in serum separator tubes, and centrifuged immediately. Serum was then stored at 0-4 °C until analysis. The samples were analyzed with Konelab 20i clinical chemistry analyzer.

Anatomopathological aspects
After 12 weeks on experimental diets, Guinea pigs were sacrificed. The liver was removed and prepared for histological procedures. Olympus CX41 microscope with camera was used for microscopic exam.

Statistical analysis
Data are presented as mean values. Statistical significance of experimental observations was determined by one-way analysis of variance (ANOVA), with the level of significance set at P<0.05.

RESULTS AND DISCUSSIONS

Body weight
At the control group, during the eight weeks of study, it could be observed an increase in body weight, which was physiological due to upgrowing animal’s age (3 months). The positive group presented a significant increase in body weight (p=0.0039) compared to control group, due to the constant administration of cholesterol in diet. Comparative, it can be observed that the mean weight at these groups have an ascendant direction, but the weight gain of the positive group in higher than at the control group (134.4 g vs 117.8 g). Significant differences (p=0.04077) were also obtained in what concerns weight gain at Omega-3 group. Even if the animal’s diet was enriched with 0.5% cholesterol, the supplementation of diet with
Omega-3 polyunsaturated fatty acids in dose of 0.6 mg/kg had an influence on their weight thus, the weight gain of this group was of 49.6 g (Tab. 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>2\textsuperscript{nd} week</th>
<th>4\textsuperscript{th} week</th>
<th>6\textsuperscript{th} week</th>
<th>8\textsuperscript{th} week</th>
<th>Weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>414.2</td>
<td>471.8</td>
<td>499.6</td>
<td>504.2</td>
<td>532</td>
<td>117.8</td>
</tr>
<tr>
<td>High fat diet group</td>
<td>441.4</td>
<td>467.8</td>
<td>517</td>
<td>538</td>
<td>576</td>
<td>134.4</td>
</tr>
<tr>
<td>Omega-3 group</td>
<td>459.4</td>
<td>447.4</td>
<td>471.4</td>
<td>495</td>
<td>509</td>
<td>49.6</td>
</tr>
</tbody>
</table>

* Values are given as mean, in grams

Serum parameters

The data from biochemical determination are presented in table no 2. The values of the control group are ranging between the minimum and maximum limits of this specie (TC: 20-80 mg/dl, TG: 0-125 mg/dl, LDL-C: 35-70 mg/dl and HDL-C: 5-20 mg/dl).

At positive group it can be observed that at the 2\textsuperscript{nd} and the 3\textsuperscript{rd} determination, the total cholesterol (89.5 mg/dl and 89 mg/dl) and triglyceride’s (129 mg/dl and 169.5 mg/dl) values are not ranging between normal limits, exceeding the maximum value, which is due to administration of successive doses of cholesterol. A low HDL-C level (4 mg / dl at baseline and 9 mg / dl in the 8\textsuperscript{th} week) is also a factor of major cardiovascular risk, independent of other lipid fractions, but also a risk factor that contributes to the changing of the LDL-C therapeutic targets whose values are increasing, reaching the value of 47 mg / dl. From biochemical results, it can be sustained that the positive group is prone to cardiovascular diseases, which are in direct relationship with obesity. Statistical analysis indicate a significant increase in total cholesterol (P=0.00950), triglycerides (P=0.00001) and LDL cholesterol (P= 0.00134) when compared to control group.

The evolution of biochemical parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4\textsuperscript{th} week</td>
<td>8\textsuperscript{th} week</td>
<td>Baseline</td>
</tr>
<tr>
<td>Control group</td>
<td>51</td>
<td>58.5</td>
<td>65</td>
<td>82</td>
</tr>
<tr>
<td>High fat diet group</td>
<td>48</td>
<td>89.5</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>Omega-3 group</td>
<td>53</td>
<td>44</td>
<td>56</td>
<td>85</td>
</tr>
</tbody>
</table>

* Values are given as mean, in mg/dl

The Omega-3 group biochemical parameter’s evolution is favorable from value’s point of view. It can be observed a decrease of TG value at the third determination (40 mg/dl) beside baseline (85 mg/dl). The literature cites that in patients receiving a food supplement with Omega-3 fatty acids, decreases the amount of triglycerides up to 38% (Schmidt et al, 1990). The average value of HDL cholesterol is continuing to increase in all three
determinations from 9 mg/dl to 15 mg/dl in the 8th week. The HDL-cholesterol (HDL-C), a classical parameter of lipid balance, used among with the total cholesterol (TC), the triglycerides (TG) and the LDL cholesterol (LDL-C) is an influent factor of cardiovascular risk. Many epidemiological studies showed a positive correlation between the HDL-C level and cardiovascular protection, its increasing concentration being an important therapeutic objective. For this purpose it is important to understand the HDL-C’s role in organism and point out the favorable and unfavorable factors which may influence this circulating lipoprotein fraction. The LDL-C values remain almost constant. Compared to control group, there is a significant decrease in TC (P= 0.01), TG (P=0.00001) and LDL-C (P=0.0995).

Anatomopathological lesions

At the control group it couldn’t be observed any macroscopic alteration that can indicate obesity. At the sacrifice, all organs showed normal structure, without modifications, perirenal and retroperitoneal adipose tissues being in normal quantities. The liver presented a normal aspect (fig. 1). Microscopically it can be seen the specific liver structure and arrangement of the Remak columns converging toward the centrolobular vein. The hepatocytes have a normal shape and structure without alterations in the nucleus or cytoplasm (fig. 2).

Macroscopic changes from positive group could be seen on the surface of the liver and profoundly. Thus, the liver was enlarged, with rounded margins, yellowish color, with obvious lobulation, friable (fig. 3). The presented changes are correlated with a high intake of cholesterol.

The histological slides from the positive group presented significant histological modifications. In the liver it can be noticed the diffuse hepatosteatosis dystrophy, with large drops, that affects the hepatic lobule almost entirely, with the exception of the lobule periphery, where hepatic cells are less affected. The degenerative process begun from centrolobular vein, fact that note that the dystrophy intervened through venous path (fig. 4).
In some areas it can be seen the lipid cysts formation (red arrow), characterized by an elongated shape, clear, which appear because of the merging of some lipid vacuoles and the degeneration of the cellular membrane. Also, the cells in different stages of necrosis (green arrow) can be seen, some of which presenting an acidophilic cytoplasm and picnotic nuclei, and others in a cytolysis stage, respectively cellular lysis (fig. 5, fig. 6).

At Omega-3 group, the liver had a normal aspect, dark-red-brown color, non-friable, (fig. 7). The lobulation couldn’t be observed.

Microscopically it can be seen the lipid dystrophy with small drops (pink arrow) situated in the surroundings of the centrolobular vein as well as some areas with lipid dystrophy with large drops. Also one can see areas in which the liver’s cell architecture does not present alterations (fig. 8). In contrast to the positive group where it was found this kind of lipid dystrophy, it can be noticed the fact that here the degenerative process is of a lower intensity and smaller extension. Therefore it can be deduced that in the case of Omega – 3 group, the fatty acids administration have a certain protection, but not totally.
CONCLUSIONS

1. The administration of a hyperlipidic diet (with 0.5% cholesterol), modify guinea pig’s body weight with 37.5%.
2. High amounts of dietary cholesterol determine the increase of the biochemical parameters involved in the cardiovascular pathogenesis, especially the total cholesterol and the triglycerides.
3. The administration of 0.5% adding of cholesterol in the diet on a long term determines the morphologic alterations in the liver expressed through degenerative lesions of the hepatocytes – hepatosteatosis diffuse with large drops.
4. A daily dose of 0.6 mg/kg Omega-3 cancel the effect of dietary cholesterol concerning the body weight, the animals from Omega-3 group being at the same weight as those from the control group.
5. In what concerns the biochemical parameters, Omega – 3 influences clearly in the most obvious way the level of the triglycerides in decreasing their levels with 16.43%.
6. The hepatic protection in the case of supplementation the diet high in cholesterol with Omega-3 is not totally assured, fact proved by the presence of small drops steatosis.

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REFERENCES


