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## Obese Childhood Dyslipidemia Management Beyond Statins — MUFA, PUFA, and Sea-buckthorn Supplements

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Additional information is available at the end of the chapter

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### Abstract

The dyslipidemia pattern usually associated with childhood obesity consists of a combination of elevated triglyceridemia, decreased plasma high density lipoprotein cholesterol concentration and LDL-c concentration at the upper limit of the normal range. This type of dyslipidemia is associated with dense and small LDL, which are proatherogenic. High circulating levels of oxidized LDL were described in extreme pediatric obesity, in children with high fructose intake and are associated with insulin resistance. The worst effect on blood lipids have trans and saturated fatty acids. But the amount of total energy intake plays more important role in lipid profiles. In childhood obesity it seems that insulin resistance precedes the development of the metabolic syndrome feature and insulin resistance is correlated with dyslipidemia. Insulin resistance increases free fatty acid flux to the liver by decreased inhibition of lipolysis and also by increased de novo lipogenesis. Fish oil is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and has hypotriglyceridemic effect in comparison to monounsaturated fatty acids. Passive in utero exposure to a hyperlipidemic environment may have programmed these children for accelerated atherosclerosis. The infant formula should be enriched with long chain fatty acids because this supplementation is associated with lower blood pressure during later childhood. In obese children, supplements with Omega-3 polyunsaturated fatty acids improve lipid profile, blood pressure values and inflammatory markers. Omega-3 fatty acids prevent metabolic syndrome, by reducing hepatic steatosis, visceral fat, by reducing serum triglycerides and improving insulin sensitivity. Potentially all compounds of

the Sea buckthorn berry, including flavonols, carotenoids, fatty acids, tocopherols and phytosterols can affect the metabolic profile. Special features of the berry oils are high proportions of palmitoleic acid as well as vitamin E, carotenoids, and sterols. The palmitoleic acid stimulates muscle insulin action, suppresses hepatosteatosis and prevent the deleterious effects of saturated fatty acids and high glucose on human pancreatic beta-cell turnover and function. Phenolic compounds and flavonoids from sea buckthorn ameliorate bodyweight, blood glucose, and serum lipid profile. By reducing triglyceridemia and by improving the blood pressure levels, sea buckthorn pulp oil may prevent metabolic syndrome in obese children. The treatment is recommended in hypertriglyceridemic waist phenotype obese children. Omega-3 supplements and sea buckthorn pulp oil supplements reversed the carotid intima media thickness values in obese children and they have beneficial effects in childhood obesity

**Keywords:** MIFA, PUFA, childhood obesity

## 1. Introduction

The World Health Organization (WHO) regards childhood obesity as one of the most serious global public health challenges for the 21st century. Childhood obesity is associated with a higher chance of obesity, premature death, and disability in adulthood. But in addition to increased future risks, overweight and obese children are at an increased risk of developing various health problems. Visceral obesity leads to insulin resistance, mediated by free fatty acids and adipokines [1, 2].

The present review deals with the management of obesity and dyslipoproteinemia in childhood and emphasis the beneficial effects of supplements with Omega-3 fatty acids and Sea-buckthorn (*Hippophae rhamnoides*) pulp oil obtained by cold pressing.

## 2. Dyslipidemia in childhood obesity

The dyslipidemia pattern usually associated with childhood obesity consists of a combination of elevated triglyceridemia, decreased plasma level high-density lipoprotein cholesterol (HDL-c) concentration, and low-density lipoprotein cholesterol (LDL-c) concentration at the upper limit of the normal range. According to the nuclear magnetic resonance spectroscopy results, this type of dyslipidemia is associated with dense and small LDL, less stable HDL, a reduction in total HDL-c, and in large HDL particles [3]. Small and dense LDL particles are associated also with visceral fat and insulin resistance. In school-age children, greater total and central adiposity are associated with smaller LDL particle size and lower HDL-c plasma levels [4].

The LDL can be easily oxidized; they have low affinity for LDL receptors and they penetrate the intima [5]. The macrophages from the vessel wall take the oxidized LDL and they are transformed into foam cells, and so the process of atherosclerosis starts. GGT (Gamma Glutamyl Transpeptidase), an enzyme known to modulate the redox status of the thiol proteins, can also catalyze the oxidation of LDL from the circulation, augmenting the development of atherosclerosis [6]. In the plasma, GGT constitutes complexes with albumin and lipoproteins [7]; while in the atheromatous plaques, GGT is colocalized with oxidized LDL and foam cells [8].

HDL inhibits LDL oxidation. The antioxidant activity of HDL can be explained by its proteins, which link transitional metals, and by two intrinsic enzymatic systems: acetylhydrolase and paraoxonase [9].

High circulating levels of oxidized LDL were described in extreme pediatric obesity [10] in children with high fructose intake [11] and are associated with insulin resistance [12].

More than half of the obese children have dyslipidemia. Improper dietary habits, such as fast foods and snacks, rich in saturated and trans-fatty acids have an important contribution for dyslipidemia. Decreased physical activity and unhealthy eating habits are noticed to have higher incidence in adolescents. These lifestyle modifications, increased susceptibility to insulin resistance, and hormonal changes make pubertal subjects prone to metabolic syndrome. Dyslipidemia present in metabolic syndrome (hypertriglyceridemia and low HDL-c) is associated with insulin resistance, with inflammatory markers (C reactive protein) and with a protrombotic status [13, 14, 15].

In a recent study [16] done on 139 children, the prevalence of dyslipidemia among overweight and obese children was 50.4%. Dyslipidemia patterns were: hypertriglyceridemia 31.9%, low HDL-c 29.7%, high non-HDL-c 15.8%, hypercholesterolemia 11.9%, and elevated LDL-c 10.7%. The dyslipidemia was often (> 50%) present among those with increased waist circumference and family history of dyslipidemia.

The consumption of fructose has recently increased and it seems that in adolescents, fructose represents 12% of the total daily intake [11]. In overweight children, higher fructose intake from sweets and sweetened drinks predicts smaller LDL particle size [4].

Although the caloric intake from fat and different types of fatty acids influence the plasma lipid profile, the amount of total energy intake plays a more important role in lipid profiles [17].

The worst effect on blood lipids have trans and saturated fatty acids [18]. Partially hydrogenated vegetable oil or fish oil are very rich in trans fatty acids that increase LDL-c and decrease HDL-c, so they must be avoided in the diet. C12-16 saturated fatty acids increase both LDL-c and HDL-c levels, but stearic acid has a neutral effect on the plasma lipid profile comparable to that of oleic acid [19].

Replacement of saturated fatty acids by polyunsaturated fatty acids (PUFA) or monounsaturated fatty acids (MUFA) lowers both LDL-c and HDL-c [20].

Supplementation with MUFA is supported by authoritative bodies. When MUFA replaces saturated fatty acids it reduces total cholesterol, and when MUFA replaces carbohydrates it decreases triglycerides and increases HDL [21].

The atherogenic lipid profile is more severe in obese children, especially in boys, who are insulinoresistent. The severity of obesity estimated by the value of body mass index (BMI) is of a lesser importance in comparison with insulin resistance on the atherogenic lipid profile [22].

The atherogenic index can be calculated in many ways, but the most used procedures are: as the ratio between total cholesterol to HDL cholesterol or as a ratio apoB/apoA1. By assessing atherogenic indexes, it has been demonstrated that overweight and obese children have twice higher risk of atherosclerosis [23] and that advancing puberty and advancing age are atherogenic risk factors for obese boys [24].

The relation between plasma lipids and prediabetes in obese prepubertal children is proved by the association of saturated fatty acids in triglycerides with the HOMA-IR (homeostatic model assessment of insulin resistance) [25].

In children, the process of atherosclerosis starts at an early age and is linked to visceral obesity [26]. The common carotid artery intima-media thickness (C-IMT) measured by ultrasound imaging is a marker of preclinical atherosclerosis. C-IMT relates to the severity and extent of coronary artery disease and predicts the likelihood of cardiovascular events in adults.

True primary prevention of atherosclerosis, as contrasted with primary prevention of clinically manifest atherosclerotic disease, must begin in childhood or adolescence [27].

Results from the Young Finns Study have shown that conventional childhood risk factors, such as dyslipidemia, obesity, elevated blood pressure, and smoking, are predictive of subclinical atherosclerosis in young adults [28]. The same authors of the study underline the good news that the adverse cardiometabolic effects of childhood overweight/obesity are reversed among those who become nonobese adults.

The relation cause-effect between dyslipidemia and insulin resistance is not well established but they are interdependent. Most of the researchers consider that insulin resistance precedes the development of the metabolic syndrome feature, including dyslipidemia [29].

Insulin resistance increases free fatty acid flux to the liver by decreased inhibition of lipolysis and also by increased de novo lipogenesis [30].

Supplements with docosahexaenoic acid (DHA) reduce plasma concentrations of free fatty acids of LDL-c and the ratio triacylglycerols/HDL-c, improving insulin resistance [31].

Eicosapentaenoic acid (EPA) increases the anti-inflammatory monocyte cytokine IL-10 expression and reduces arterial stiffness, which may contribute to the antiatherogenic effect of EPA in obese dyslipidemic patients [32].

## **2.1. Effects of MUFA and PUFA supplements on dyslipidemia associated to childhood obesity**

The MUFA from n-7 and n-9 classes can be synthesized in our body from acetyl-coA, but the essential PUFA from n-3 and n-6 classes are required in the diet. A balance between n-6 and n-3 PUFA must be maintained in the diet. There is no consensus about the value of this ratio, but the most used value is around 5. Linoleic acid (C18 delta 9,12) represents the parental fatty acid for class n-6 and linolenic acids (C18 delta 9,12,15) for class n-3, respectively. Naturally, the structure of unsaturated fatty acids is cis fatty acids. PUFA are incorporated in the structure of membrane phosphatides involved in cell fluidity, permeability, and signal transduction. n-3 PUFA are important in the brain development in the fetus and also in early postnatal life [19].

Differences in the composition of dietary fat may also contribute to adipose tissue development by altering rates of adipocyte differentiation and proliferation. Relatively low intake of n-3 PUFA and excessive dietary linoleate may contribute to excessive adipose tissue [33].

Postmortem examination of fetuses delivered from hypercholesterolemic mothers demonstrated that passivity in utero exposure to a hyperlipidemic environment may have programmed these children for accelerated atherosclerosis [34].

It was demonstrated that an enhanced maternal-fetal n-3 PUFA status was associated with lower childhood adiposity [35]. But, in another study, supplementation with n-3 fatty acids during pregnancy and lactation didn't influence significantly the fat mass in the offspring during the first year of life [36]. Good news is that fish oil supplementation during lactation affects blood pressure and body composition of children [37] and that nutritional interventions may improve plasma long-chain PUFA profile and metabolic outcomes of normolipidaemic obese children [38].

According to the European directive, the infant formula should be enriched with long-chain PUFA because this supplementation is associated with lower blood pressure during later childhood. In clinical studies done in adults, fish oil supplementation gave varying results on blood pressure values but most of them showed a lowering in the blood values [19].

A decrease in serum n-3 PUFA, especially DHA, and an increase in saturated FA was noticed in obese children versus lean controls. The subcutaneous adipose tissue and not the visceral adipose tissue was correlated to the changes in PUFA and saturated FA, suggesting an abnormal essential FA metabolism in obese adolescents [39].

Fish oil is rich in EPA and DHA and has a hypotriglyceridemic effect in comparison to MUFA, but supplementation only with DHA does not share the hypotriglyceridemic effect. Fish may be more beneficial than fish oil supplementation. The favorable effect of cis-MUFA on cardiovascular diseases is unlikely, but of those n-3 PUFA is suggestive [19].

Omega-3 PUFA supplementation was associated with a reduced level for triglycerides and an up-regulated expression of the gene encoding peroxisome proliferator activated receptor- $\alpha$  (PPAR $\alpha$ ), a transcription factor that increases fatty acid oxidation and down-regulates pro-inflammatory genes [40].



Omega-3 fatty acids can prevent prediabetes and diabetes mellitus development because these PUFA are ligands for peroxisome proliferator receptor activator gamma (PPAR $\gamma$ ) involved in insulin sensitivity and also, by constituting of the novel biologically active lipid mediators (resolvins and protectins), which also increase insulin sensitivity. The obesity-induced hepatic steatosis can be prevented by Omega-3 PUFA because they decrease endogenous lipid production by inhibiting the expression of the transcription factor, sterol response element binding protein-1c, SREBP-1c [41].

In accordance with the above molecular effects, beneficial effects of Omega-3 PUFA in obesity were demonstrated in clinical and experimental studies. It was shown that Omega-3 fatty acids prevent metabolic syndrome by reducing hepatic steatosis and visceral fat, by reducing serum triglycerides, and improving insulin sensitivity [42, 43].

Omega-3 fatty acids, by inhibiting hepatic lipogenesis, reduce a jéun and postprandial triglyceridemia [44] and improve the quality of platelets membrane phospholipids. In extrahepatic tissues, the activation of lipoprotein lipase has also a great contribution to the hypotriglyceridemic effect of Omega-3 fatty acids. They have also an antiatherogenic effect by reducing the quantity of small and dense LDL [45].

Many clinical and experimental studies have demonstrated that omega-3 PUFA have lipid-lowering effects. The improvement in lipid profile is due mainly to enhanced fatty acid beta-oxidation and suppression of fatty acid synthesis in the liver [46, 47, 48, 49].

During PUFA treatment, the gene expression of the lipogenesis enzyme sterol regulator element binding protein-1c (SREBP-1c) is decreased while the fatty acid oxidation in the liver is increased via the activation of peroxisome proliferator activated receptor- $\alpha$  (PPAR- $\alpha$ ) [50, 51].

Dyslipidemia and insulin resistance are associated with non-alcoholic fatty liver disease (NAFLD). Diet rich in lipids and augmented lipolysis in adipose tissues increase the hepatic pool of fatty acids for the liver. Hyperglycaemia and hyperinsulinemia increase lipogenesis in the liver and contribute to hepatic steatosis [52, 53].

Omega-3 PUFA have beneficial effects in liver steatosis by decreasing triglyceridemia and by reducing the muscle intramyofibrillar triglycerides [51].

By upregulating the genes involved in insulin sensitivity, namely glucose transporters (GLUT-2/GLUT-4), peroxisome proliferator activated receptor- $\gamma$  (PPAR- $\gamma$ ), and insulin receptor signaling (IRS-1/IRS-2) [41], Omega-3 PUFA increase insulin sensitivity.

Lipid intake should represent 25–35% of total energy intake. In USA, saturated FA represents 11% of total energy intake, PUFA 7%, and MUFA 12%. Excess consumption of fatty acids, above the intake recommended, lead to weight gain that is detrimental for body health [21].

When in the diet, saturated fatty acids and trans fatty acids are replaced by cis-MUFA and PUFA, plasma levels for total cholesterol and LDL-c are reduced, while HDL-c concentration remains unchanged. According to the value of the calculated atherogenic index (total cholesterol/HDL-c), this should mean a beneficial effect. Diets rich in cis-MUFA and PUFA may

improve insulin sensitivity; and cis-MUFA compared with carbohydrate and saturated fatty acids reduced HOMA-IR values [19].

When replacing carbohydrate and saturated fat, MUFA consumption can be beneficial. MUFA has positive impact on surrogate markers, but the potential impact on disease outcome remains unclear. When MUFA replaces saturated fatty acids it reduces total cholesterol and when MUFA replaces carbohydrates it decreases triglycerides and increases HDL [21].

Nowadays, the recommendation is to replace solid fats with oils rich in MUFA and PUFA and the most recommended diet is the Mediterranean diet. A randomized controlled trial done on 7,000 patients demonstrated that Mediterranean diet beats low-fat diet. In the study, the two groups of subjects with Mediterranean diet that were supplemented with either olive oil (approximately 4 tablespoons/day) or nuts (average of 3 servings/day) versus the group with low-fat diet reduced the risk for major cardiovascular events [54].

## **2.2. Effects of supplements with Sea-buckthorn fractions on dyslipidemia associated to childhood obesity**

Sea-buckthorn (*Hippophae rhamnoides*) is a plant of seashores and cliffs; it can be found in Central Asia and Europe, all the way from the shores of the Black Sea to the northwestern shores of the continent. In addition, Sea-buckthorn has spread to Canada and the United States and nowadays the plant has been currently domesticated. Both the berries and the leaves of the plant can be used as dietary supplements.

According to Greek legend, the sick and wounded horses of warriors would be allowed to roam and feed on the shrub. The horses would return more strong and healthy. Sea-buckthorn is a special food that is used to help astronauts maintain their health in space [55].

Since ancient times, Sea-buckthorn berries have been used for their beneficial effects on blood circulation, skin regeneration, and anti-inflammatory effects. The berry contains oil, both in the seed and in the soft parts (pulp oil from the flesh and peel). Special features of the oils are high proportions of palmitic (16:0), oleic (18:1n-9), palmitoleic (16:1n-7), linoleic (18:2n-6), and  $\alpha$ -linolenic (18:3n-3) acids as well as vitamin E, carotenoids, and sterols [56].

While the seed oil is rich in linoleic and  $\alpha$ -linolenic acids, the pulp oil is a very rich source of palmitoleic acid. This acid is an essential fatty acid that is rare to find in the natural form. Macadamia nuts are the only other source of Omega-7 fatty acids and only in trace amounts [57].

The pulp oil provides a 1:1 ratio of Omega-3/Omega-6. The pulp can be consumed either as a juice or a puree. All the components of the berry contain flavonoides, but the seed residues and the leaves are the most important sources of flavonoides (particularly quercetin, isorhamnetin, kaempferol) [58]. Flavonoides and vitamin C have anti-inflammatory effects and act through a synergic mechanism [59].

The Sea-buckthorn fruit is a very rich source of vitamin C (695 mg per 100 grams), about 12 times greater than oranges, placing Sea-buckthorn fruit among the most enriched plant sources of vitamin C [60].



The origin (subspecies of more than seven) and harvesting time of the berries, as well as oil isolation technology, influence the oil composition [61]. The amounts of bioactive compounds in sea buckthorn berry vary depending on the subspecies, area and year of cultivation, and the maturity of the berries [62].

The method used for extracting Sea-buckthorn influences the potency and the quality of the oil. Cold pressing, hot pressing, solvent extraction, and maceration in other carrier oils are the most used ways for Sea-buckthorn oil extraction, but each method have its own disadvantages. For example, cold-pressing may be ideal, but the extraction rate is quite low. Hot-pressing destroys healthy nutrients and solvent extraction may contaminate the oil with hazardous solvents. The best way to obtain Sea-buckthorn oil is through supercritical CO<sub>2</sub>-extraction.

Potentially all compounds of the berry, including flavonols, carotenoids, fatty acids, tocopherols, tocotrienols, and phytosterols of the pulp oil, can affect the metabolic profile. Clinical and experimental studies have demonstrated a wide range of positive effects of the sea buckthorn oils and flavonoides on the lipid profile. Animal and in vitro studies have suggested that sea buckthorn oils [63, 64, 65] and alcohol extracts and flavonoid preparations [66, 67, 68, 69] have antioxidant and anti-inflammatory activities and may beneficially affect serum glucose and triglyceride concentrations. The effect was observed in participants who had a metabolic profile that reflected higher cardiometabolic risk.

In two clinical studies [70, 71] involving healthy men, the intake for 8 weeks of Sea-buckthorn juice versus placebo or supplementation with 5 g of Sea-buckthorn berry oil for 4 weeks versus coconut oil control had no significant influence on the lipid profile (total cholesterol, LDL-c, HDL-c, and triglycerides), but decreased moderately the susceptibility of LDL to oxidation [71].

In healthy, normolipidemic adults having healthy diets, consumption of Sea-buckthorn berry did not affect the circulating concentration of lipid markers, but increased the fasting plasma concentration of quercetin and isorhamnetin indicating that Sea-buckthorn berry is a good dietary source of flavonols [72].

There is a paucity of clinical and animal experiments focused on Sea-buckthorn oil effects on dyslipidemia associated to obesity and the studies are lacking in childhood obesity. Also, in some published data, the tested oils are not defined and some studies should have a better design. The influence of the oils on the plasma lipid levels needs further investigation.

In the experimental study done on white albino rabbits fed with high cholesterol, CO<sub>2</sub> extracted Sea-buckthorn seed oil treatment (1 ml for 30 days) had significant anti-atherogenic and cardioprotective activity. The treatment increased the HDL-c plasma levels and restored the acetylcholine-induced vasorelaxant effect to that of normal values [73].

A randomized, double-blind, crossover study including two 4-week periods with either 3 g/day of black currant seed oil or 2.8 g/day of fish oil separated by a 4-week washout period was done on 15 healthy females. The results showed that black currant seed oil had minor changes on serum n3 fatty acids. Serum levels of LDL cholesterol were lower ( $p < 0.05$ ) after black currant seed oil compared to fish oil. Plasma glucose concentration decreased during the

fish oil supplementation ( $p < 0.05$ ). The results underline the beneficial effects of berries and berry seed oils on serum lipid profile [74].

In a study group including 49 atopic dermatitis patients who took 5 g (10 capsules) of seed oil, pulp oil, or paraffin oil daily for 4 months, a significant ( $p < 0.05$ ) increase in the level of high-density lipoprotein cholesterol from 1.38 to 1.53 mmol/L was observed in the pulp oil group [75].

It was demonstrated that a high-MUFA, cholesterol-lowering diet may be superior to a low-fat diet because it lowers triglyceridemia and does not decrease the plasma level of HDL cholesterol [76].

C16:1n7-palmitoleate is known as an adipose tissue-derived lipid hormone that works at the crosstalk between lipid and sugar metabolism. The acid stimulates muscle insulin action and suppresses hepatosteatosis [77]. The following lines will present the effects of palmitoleic acid on insulinodependent tissues.

In vitro studies done on rat L6 skeletal muscle cells, it was demonstrated that palmitoleic might facilitate uptake and utilization of glucose by upregulation of the activities of the glucose transporters GLUT1 and GLUT4 [78].

On a spontaneous model of obese type 2 diabetes rats, researchers demonstrated that repeated administration of palmitoleic acid increased insulin sensitivity by down-regulating mRNA expressions of proinflammatory adipocytokine genes (TNF $\alpha$  and resistin) in white adipose tissue and by decreasing hepatic lipid accumulation. Palmitoleic acid down-regulates the mRNA of lipogenic genes (as an example, SREBP-1) in the liver and reduces both the plasma triglyceride and hepatic triglyceride levels [79].

It is worth mentioning the effects of C16, n-7 on pancreatic tissue. It was demonstrated that palmitoleic and oleic acids prevent the deleterious effects of saturated fatty acids and high glucose on human pancreatic beta-cell turnover and function via Bcl-2 [80].

In a recent randomized crossover study, 80 overweight women were divided into four groups with different supplements intake for 30 days: dried Sea-buckthorn berries, Sea-buckthorn oil, Sea-buckthorn phenolics ethanol extract mixed with maltodextrin (1:1), or frozen bilberries. Sea-buckthorn-induced effects were mainly on serum triglycerides and very-low-density lipoprotein (VLDL) and its subclasses in the groups with higher metabolic risk. From the supplements, Sea-buckthorn oil induced a decreasing trend in serum total, IDL, and LDL cholesterol and apolipoprotein B in participants with the baseline metabolome of higher cardiometabolic risk [81].

In the Sea-buckthorn, most antioxidants appear to accumulate in the seeds relative to the pulp, leaves, or stem despite most flavonoids being in the leaves. The total phenolic content of the leaves is high and is between 47.06–66.03 mg/g rutin equivalents (RE) [82].

Phenolic compounds and flavonoids ameliorate bodyweight, blood glucose, and serum lipid profile. Also flavonoids from seeds and leaves have anti-obesity and hypoglycemic effect.

Clinical studies have demonstrated that the berry and the ethanol fraction from Sea-buckthorn pulp has beneficial effects on postprandial glucose and insulin levels [83].

Experimental studies done in diabetic rats demonstrated that flavonoids present in water extracts of Sea-buckthorn seeds have hypoglycemic and triglyceride-lowering effect [84].

The fibers and polyphenols in Sea-buckthorn (*Hippophaë rhamnoides*) extraction residues delay also the postprandial lipemia [85].

Quercetin, as an important flavonoid in the Sea-buckthorn, has also hypolipidemic effect [86].

Quercetin inhibits fatty acid and triacylglycerol synthesis in rat liver cells [87].

In vitro studies demonstrated that quercetin and isorhamnetin have protective effects against oxidized LDL-induced endothelial cell injuries. The flavonoids' beneficial effects might derive from their antioxidant activity and from their capability in modulating the expression of eNOS (endothelial nitric oxide synthase) and LOX-1 (lipooxygenase-1) [66].

There are some molecules that are (currently known to be) unique to Sea-buckthorn named flavonoid glycosides [88] and they have antioxidant activity.

High-fat diet obese C57BL/6J mice treated with 70% ethanolic extract of Sea-buckthorn at 500–1,000 mg/kg bodyweight over 13 weeks had lower hepatic and serum total cholesterol, lower hepatic triglycerides and serum leptin level versus non-treated mice. Triglyceridemia and insulinemia were similar in the studied group. The study demonstrated that the Sea-buckthorn intervention was effective in preventing body weight gain and fat accumulation in the liver. The molecular mechanism of this effect is the increase of the hepatic mRNA expression of peroxisome proliferator-activated receptor (PPAR)  $\alpha$  and PPAR- $\gamma$  while the level of the hepatic key enzyme in the fatty acid synthase was decreased [89].

Many compounds from Sea-buckthorn help lose extra weight. Omega-7 in Sea-buckthorn sends messages to the brain, telling it to stop storing calories as excess fat. Sea-buckthorn oil stimulates healthy bowel moves, thereby protecting cell membranes from oxidative and physical stress.

In overweight or obese women, the intake of different berries and berry fractions for 33 days with washout periods of 30–39 days have resulted in positive effects such as a significantly decrease in the waist circumference and in the level of vascular cell adhesion and intercellular adhesion molecules [90].

### **2.3. Association of the atherogenic indexes with antropometric, inflammatory, oxidative stress and endothelial dysfunctional markers in childhood obesity**

It was demonstrated that in obese children with metabolic syndrome, there is a positive association between the high waist circumference and the atherogenic index (total cholesterol/HDL-c) [91].

Endothelial dysfunction, inflammation, and oxidative stress are present in childhood obesity. Especially during puberty, there are some pro-inflammatory and pro-oxidative changes

associated with a relative insulin resistance. The association of the inflammatory and oxidative stress markers with the high value of the apo B/apo A1 ratio in obese children underlies the action in the cluster of different pathogenic mechanisms, augmenting the atherosclerosis development [26, 92].

Apolipoproteins B and A-1 are proposed as markers with value in pediatric lipid risk assessment. High apo B and low apo A-1 levels, usually present in obese children and adolescents reflect a lipoprotein profile predisposing to the development of subclinical atherosclerosis in adulthood [93], [94].

The concentrations of plasma apolipoprotein (apo) B are often increased in childhood obesity, partly due to the hepatic overproduction of apo B containing lipoproteins [95,96].

Many researchers consider apo B as a better predictor of vascular risk than LDL because apo B, in comparison with LDL, is more strongly associated with other cardiovascular risk factors [97].

### 3. Diet and weight loss

A comparative study between severely obese children versus moderately obese children demonstrated a markedly more unfavorable cardio-metabolic risk profile in the first group. The study highlights that severely obese children need to receive particular attention regarding obesity treatment [98].

A recent study demonstrated that the strongest negative predictor of weight loss is waist circumference. The study underscores that obese children with abdominal fat distribution need more intensive interventions [99].

In a review about the impact of dietary and physical activity in obese children, including the studies that have been done in the last 35 years, demonstrated that diet-only and diet-plus-exercise interventions resulted in weight loss and metabolic profile improvement. Diet-only intervention reduced triglycerides and LDL levels, while diet-plus-exercise interventions reduced fasting glycaemia and insulinemia and increased HDL-c concentration [100].

In obese children and adolescents, weight loss improved the values of the parameters included in the metabolic syndrome criteria with the exception of HDL-c concentration. The reduction in fasting triglycerides concentration (but not waist circumference) was the only significant predictor of metabolic syndrome change [101].

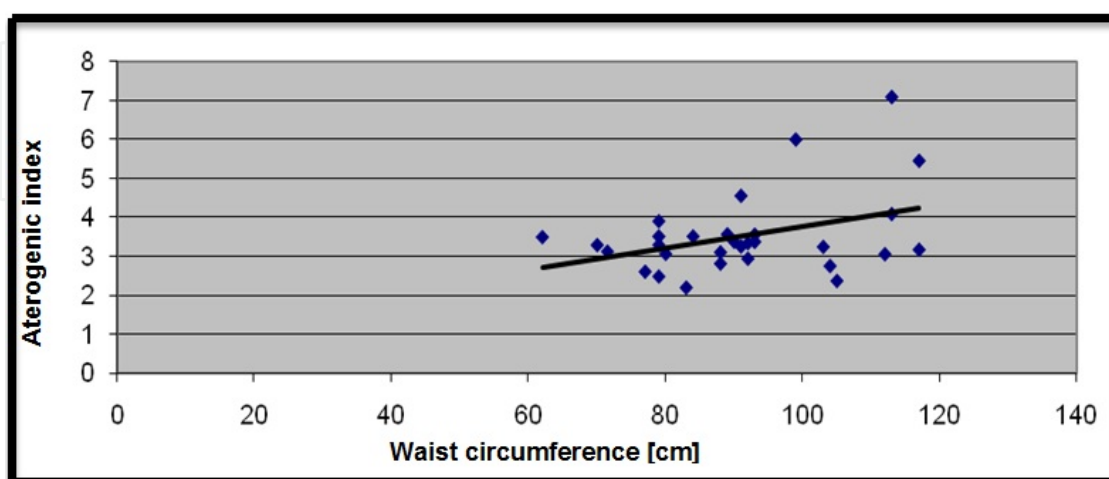
Diet modifications and physical activity have been included in obesity management, but have shown relatively limited success among severely obese children and adolescents. However, the parents of obese children should know that a healthy lifestyle is important for better physical and mental health no matter how much or how little weight is lost. The physician should help patients to cope obesity for psychosocial functioning and must motivate them to make use of the available healthcare resources.

Pharmacotherapy for obesity has side effects (growth problems, lessened self-esteem, unhealthy weight-control mechanisms) and frequently fails to be efficacious because obesity is a multifactorial, polygenic disease [102,103].

#### 4. Own results

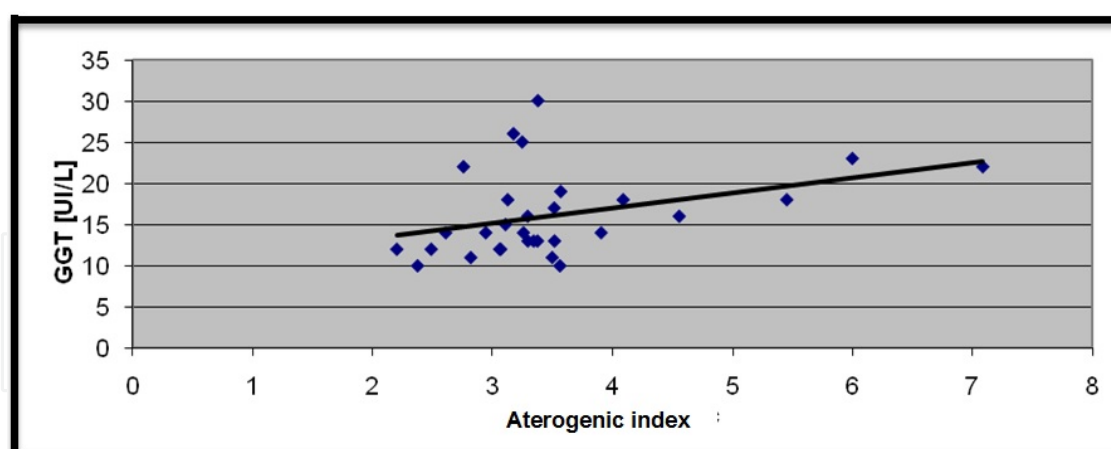
We did some studies in obese children and adolescents ( $n = 41$  and  $n = 48$ ) and analytical evaluations were performed before and immediately after supplements were administered. In one study we gave Sea-buckthorn pulp oil (800 mg/day for 60 days) and placebo, and in another study we gave Omega-3 fatty acids (DHA 130 mg and EPA 25 mg) associated with vitamins (A 200  $\mu\text{g}$ , D 1.25  $\mu\text{g}$ , E 2.5 mg, and C 30 mg). As a control, we enrolled 30 lean children. All the children under medications or those with chronic disease (endocrine, inflammatory, or hereditary diseases) or smokers were excluded. The participants were instructed not to change their lifestyle (dietary and drinking habits, physical activity) during the whole studies and not to take any additional medication including vitamin supplements.

The atherogenic indexes (calculated either as total cholesterol/HDL-c or apoB/apo-A1) were calculated in obese children. Higher values for atherogenic indexes have predictive values for atherosclerosis development. The atherogenic indexes were correlated with other cardiovascular risk factors such as: waist circumference (Figure 1), C reactive protein (CRP), GGT activity (Figure 2), diastolic blood pressure, and malondialdehyde (MDA)-marker of lipid peroxidation (Figure 3). The multiple associations of atherogenic index with dyslipidemia, anthropometric, inflammatory, and oxidative stress markers underline that in obesity there is a cluster of pathogenic mechanisms that contribute to atherosclerosis. Also, the atherogenic indexes were associated with subclinical atherosclerotic disease markers as carotid intima media thickness-CIMT (positive correlation) (Figure 4) and with brachial artery flow-mediated dilation-FMD (Flow Mediated Dilatation (negative correlation) (Figure 5) [104, 105].



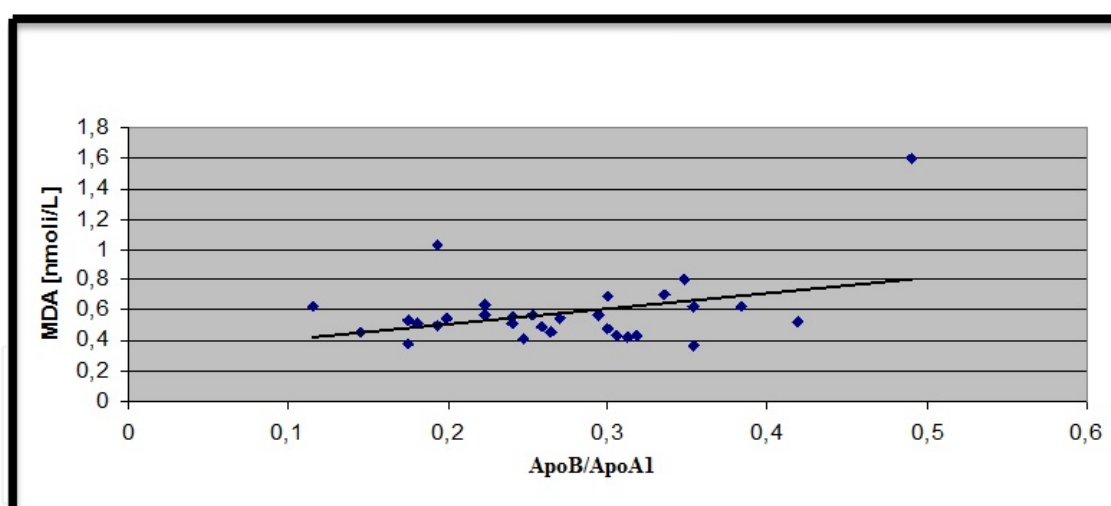
**Figure 1.** Correlation between atherogenic index and waist circumference ( $r = 0.33$ ). The atherogenic index was calculated as the ratio between total cholesterol/HDL-c.





**Figure 2.** Correlation between GGT activity with atherogenic index ( $r = 0.42$ ). The atherogenic index was calculated as the ratio between total cholesterol/HDL-c.

By hydrolyzing the glutathione, the GGT has a pro-oxidant effect. Also, the products of the GGT reaction may themselves lead to increased free radical production, particularly in the presence of iron. High serum GGT activity was positively associated with the risk of coronary heart disease, type 2 diabetes mellitus, and stroke. We have demonstrated a positive correlation between GGT activity and the cholesterol/HDL-c ratio value [104] (Figure 2).



**Figure 3.** Correlation between MDA and apoB/apo A-1 ( $r = 0.39$ ).

There is a paucity of studies on systemic oxidative stress in childhood obesity but almost all of them have demonstrated higher levels of serum malonyldialdehyde, a marker of lipid peroxidation. There is a direct relation between dyslipidemia and lipid peroxidation and both contribute to a pro-inflammatory phenotype in childhood obesity [105, 106, 107] (Figure 5). NADPH oxidase activity (respiratory burst) in monocytes is increased in childhood obesity and together with serum lipid peroxidation contributes to an increased systemic oxidative stress [105].

Our research team demonstrated that in obese children versus lean ones, plasma total cholesterol and triglycerides were higher, while HDL-c level was lower. LDL-c concentrations were similar in the studied groups. The treatment (800 mg/day, for 60 days) with Sea-buckthorn pulp oil (obtained by cold pressing) reduced significantly the total plasma cholesterol, the apo B/apo A-I ratio and the plasma triglycerides. A weak improvement of HDL-c and LDL-c levels was noticed [105]. We consider that the composition of the Sea-buckthorn pulp oil rich in MUFA and phytosterols is responsible for this effect [108].

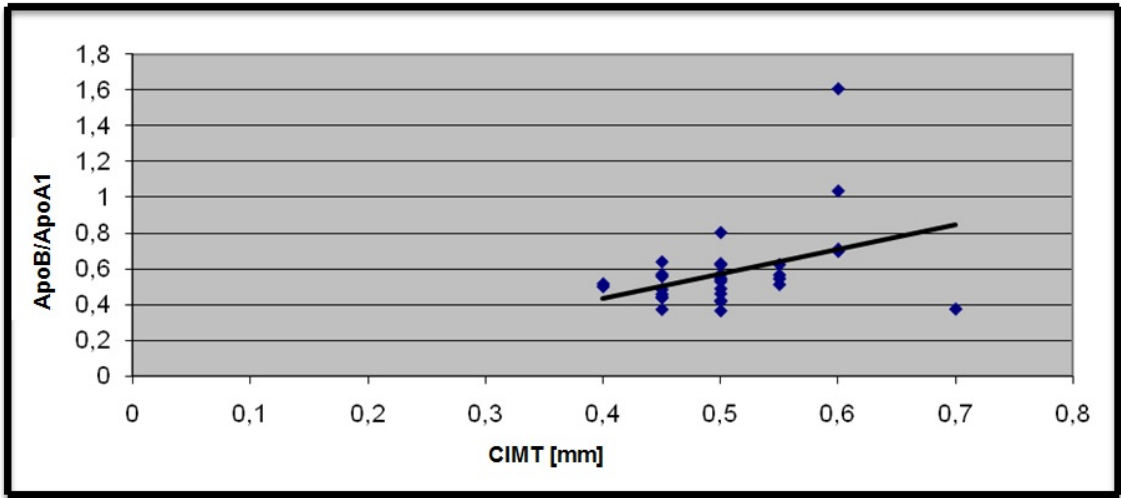


Figure 4. Correlation between apo B/apo A-1 with CIMT (r = 0.38).

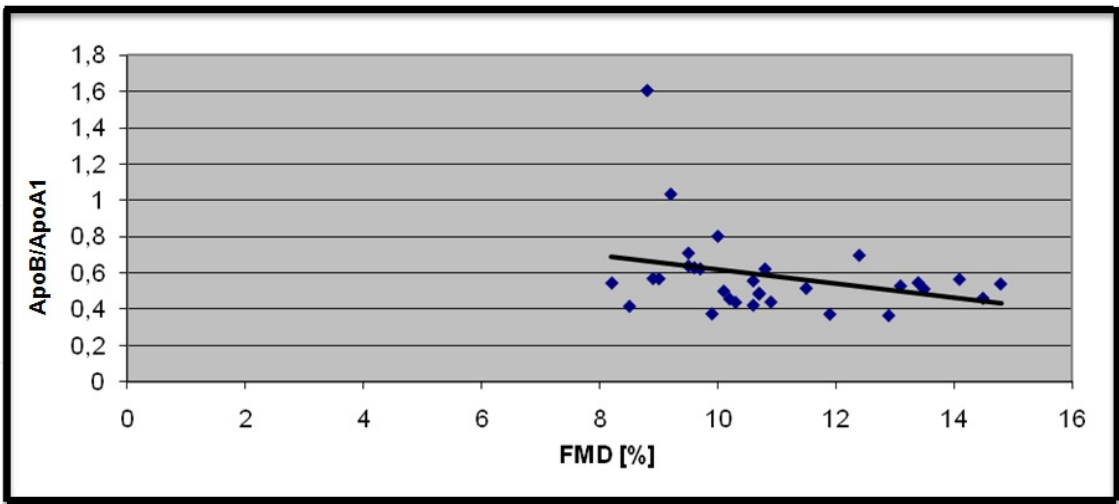


Figure 5. Correlation between apo B/apo A-1 with FMD (r = -0.36).

Dyslipidemia, inflammation, insulin resistance, and oxidative stress are important culprits for atherosclerosis in obesity. Hyperleptinemia and hypoadiponectinemia represent links between inflammation and vascular dysfunction in obese children. Atherosclerosis begins with endothelium dysfunction and insulin resistance is an important trigger. Puberty alerts

some of the inflammatory markers associated with endothelial dysfunction in obese children and leptin concentration rises. Leptin is a biomarker of vascular dysfunction, while adiponectin improves endothelial cells function. In our study done on 48 obese children, the intake of Omega-3 fatty acids (DHA 130 mg and EPA 25 mg) associated with vitamins (A 200 µg, D 1.25 µg, E 2.5 mg, and C 30 mg) for 3 months has resulted in improved lipid profile. The values for total cholesterol, LDL-c, and triglycerides were decreased, while the HDL-c level was increased [109]. We showed that in the obese children, supplements with Omega-3 fatty acids improved not only the lipid profile, but also the blood pressure values and inflammatory markers. By lowering leptin, increasing adiponectin, and by decreasing the HOMA-IR values, Omega-3 PUFA reduces the risk of cardiovascular disease development in adulthood [109].

According to the ATP III (adult treatment panel) modified criteria [110] in our studies, more than 55% of the obese children had triglyceridemia higher than 100 mg/dl and triglyceridemia was correlated with ALT (Alanine aminotransferase) activity. Our results are in accordance with others. In a study done on 700 overweight and obese children aged 7–18 years, ALT activity was correlated with BMI, waist circumference, blood pressure values, triglyceridemia, and insulin resistance [111]. Calcaterra et al. proposed the high value of ALT as a screening marker for metabolic syndrome in obese children [112].

In one of our studies, we divided the obese children in two groups: hypertriglyceridemic waist phenotype (n = 17) and obese nonhypertriglyceridemic (n = 24). Modified ATP III cut points for serum triglycerides ( $\geq 110$  mg/dL) and waist circumference ( $\geq 90$ th percentile for age and sex) were used to divide obese children. Metabolic and inflammatory parameters were measured before and after Sea-buckthorn pulp oil intake (800 mg/day, 60 days) and the best improvement of the measured plasma variables was observed in hypertriglyceridemic waist phenotype obese children. We demonstrated that high serum values for triglycerides were associated with low values for HDL-C and the treatment reduced significantly the levels of triglycerides and improved the HDL-C concentration [113].

Hepatic de novo lipogenesis is augmented in hypertriglyceridemic waist phenotype obese children and this can be demonstrated by the high values of ALT. In our study, treatment with Sea-buckthorn pulp oil reduced ALT activity and triglyceridemia and improved blood pressure levels. By reducing triglyceridemia and by improving the blood pressure levels, Sea-buckthorn pulp oil may prevent metabolic syndrome in obese children [105, 113]. The study underlines that treatment with Sea-buckthorn pulp oil should be recommended in hypertriglyceridemic waist phenotype obese children. Also, in obese children, the intake of Omega-3 fatty acids (DHA 130 mg and EPA 25 mg per day) associated with vitamins (A 200 µg, D 1.25 µg, E 2.5 mg, and C 30 mg) for 3 months reduced the blood pressure values (systolic and diastolic) significantly [109].

Some of the most important effects for Omega-3 supplements and Sea-buckthorn pulp oil supplements are to prevent atherosclerosis and to reverse the CIMT values [105,109].

In a high-fat diet mouse model, we induced non-alcoholic fatty liver disease to NMRI mice and the treatment with normocaloric/normolipidic diet and Omega-3 fatty acids (DHA 130 mg

and EPA 25 mg per day) had reversed liver histopathological results from steatohepatitis to normal aspect and improved the plasma lipid profile [114].

## 5. Treatment of dyslipidemia associated to childhood obesity

Both in adults and in children, the extent of atherosclerotic lesions is significantly correlated with a modified serum lipid profile (increased total and LDL cholesterol, triglycerides, low HDL cholesterol) together with elevated blood pressure and waist circumference.

Reversal of vascular functional abnormalities with early therapy with statins and supplementation with antioxidant vitamins and Omega-3 fatty acids has been observed in children with familial hypercholesterolemia.

According to an AHA Scientific Statement, “LDL cholesterol lowering drug therapy is recommended only in those children  $\geq 10$  years of age whose LDL cholesterol remains extremely elevated after an adequate 6- to 12-month trial of diet therapy. Drug therapy was to be considered for children with LDL cholesterol levels  $\geq 190$  mg/dL and in those with LDL cholesterol  $\geq 160$  mg/dL together with either the presence of  $\geq 2$  other cardiovascular disease risk factors or a positive family history of premature cardiovascular disease” [34].

Omega-3 PUFA represents the first choice in treating hypertriglyceridemia in childhood obesity because they do not have adverse reactions, they are safe, and they have good tolerance. Also, different Sea-buckthorn fractions do not have side effects [110–86] and they have great future as food supplements in preventing obesity complications. Future research work will show if the beneficial changes of the supplements revert back if the treatment is stopped.

Cook and Kavey recommend that “any medication except Omega-3 fish oil in youths with combined dyslipidemia should be undertaken only with the assistance of a lipid specialist” [3].

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