Dietary approach in the prevention and treatment of NAFLD

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1. ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a rapidly emerging chronic liver disorder and will increasingly be a critical global health problem. Diet is an important pathogenic factor of NAFLD and it is well documented that the increased prevalence of NAFLD during the last decades was associated with deep modification of dietary habits, especially increased intakes of fats and simple carbohydrates. As the disease stems from excess calorie intake and lack of physical activity, the correction of unhealthy lifestyles is the basis of any prevention and treatment strategy while drugs should remain a second-line of treatment. NAFLD patients should receive counselling for a low carbohydrate and low saturated fat diet, avoidance of fructose-enriched soft drinks and increased consumption of fruits and vegetables. The use of functional foods in NAFLD has been poorly studied and up to day only few reports are encouraging and incite the promotion of functional food approach on NAFLD prevention on the basis of food and nutritional genomics.

2. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a rapidly emerging chronic liver disorder and will increasingly be a critical global health problem.

NAFLD represents a spectrum of liver diseases that ranges from simple hepatic steatosis to a more severe and treatment resistant stage called non alcoholic steatohepatitis (NASH). NASH features steatosis plus inflammation and fibrosis, which may in turn progress to cirrhosis, hepatocellular carcinoma and sub-acute liver failure (1).

NAFLD, which is indistinguishable in terms of alcohol-related histological forms, is present in patients without or with minimal alcohol intake (< 30 g in males and < 20 g in females) (2).

NAFLD is characterized by an aberrant accumulation of triglycerides in the parenchymal liver cells as a result of abnormal fatty acid metabolism, including:

failure of the synthesis/secretion of apolipoproteins and triglyceride; excessive delivery of fatty acids (FFAs) and increased mitochondrial lipid oxidation (3).

3. EPIDEMIOLOGY, PATHOGENESIS AND NATURAL HISTORY

3.1. Prevalence and incidence

NAFLD is the most frequent chronic liver disease in Western countries both in adults and children (4). It is estimated that the prevalence is approximately 25%, even if it varies according to age, gender, and ethnicity (5). The incidence is two new cases/100 people/years while the 2-3% of individuals of the general population suffer from NASH (6).

The prevalence of NAFLD is higher in males, increases with age, and it is expected to increase in the following decades in association to the increase of metabolic disorders and the sedentary habits of populations of developed countries (7).

The prevalence of NAFLD among children is 3-10%, rising up to 40-70% among obese children (8). The prevalence of NAFLD is 80-90% in obese adults, 30-50% in patients with diabetes and up to 90% in patients with hyperlipidemia (9).

3.2. Pathogenesis

NAFLD is a clinical condition associated with Metabolic Syndrome (MS), a complex entity widely present in the general population, which is characterized by the presence of increased waist circumference and 2 or more of the following conditions: 1) hypertriglyceridemia, 2) hypertension, 3) high fasting glucose and 4) a low high-density lipoprotein level. Insulin resistance is the pathogenetic denominator that links all components of the metabolic syndrome and is also recognized as the most common risk factor for the development of NAFLD. From this point of view, NAFLD is considered as the hepatic manifestation of the metabolic syndrome. Furthermore, hepatic steatosis may precede the onset of metabolic syndrome and should be considered as the trigger of MS rather than its target (10) (11).

Despite the worldwide effort in the pathogenesis of NAFLD, the mechanism underlying the liver damage remains largely unknown.

According to a two-hit hypothesis, increased intrahepatic triglyceride accumulation is followed by a second step, which may lead to NASH. The net retention and accumulation of free fatty acid and triglycerides in the liver (the first hit) is the consequence of different and concomitant mechanisms such us: increased influx of free fatty lipid (as a consequence of insulin resistance), increased ingestion of dietary fats or carbohydrates, increased *de novo* lipogenesis, impaired hepatic β-oxidation of fatty acid and impaired triglyceride export. It is believed that the accumulation of fat in hepatocytes makes the liver more susceptible to adverse factors. At this point, steatosis is potentially reversible and does not necessarily lead to permanent hepatic injury (12). The "second hit" includes an imbalance of oxidative stress and lipid peroxidation of fatty acids; impaired mitochondrial and peroxisomal oxidation of fatty acids and the release of inflammatory cytokines, chemokines, and adipokines with the consequence of lobular inflammation, hepatocyte necrosis, apoptosis and cell dropout. The final result is a necroinflammatory hepatisis (NASH) that can lead to significant fibrosis (13) (14).

Recently, an alternative pathogenetic mechanism has been hyphotized. The new model suggests that many hits act equivalently, resulting in liver inflammation. In particular, dietary gut-derived and adipose tissue-derived factors (pro-inflammatory adipocytokine, IL-6 and TNFα) may play a central role in determining liver inflammation that may precede steatosis in NASH (15).

3.3. Natural history

The natural history of NAFLD is still not well defined; however, it is known that the majority of individuals with steatosis do not develop NASH and, simple steatosis will usually follow a benign course (16). On the contrary, patients with non-alcoholic steatohepatitis have an increased risk of mortality related to liver related-events (liver failure, complications of portal hypertension, HCC) and cardiovascular diseases (17).

Although an indirect association between NAFLD and cardiovascular diseases is expected, given the close association between NAFLD and classical cardiovascular risk factors (obesity, diabetes, hypertension and dyslipidaemia) a growing body of evidence supports a direct role for NAFLD in the pathogenesis of atheromatous cardiovascular diseases. The mechanism of any direct effect of NAFLD on cardiovascular risk remains unclear; therefore, possibilities include the release of atherogenic inflammatory cytokines and pro-coagulant factors from the steatotic liver (18).

4. ROLE OF DIETARY COMPONENT IN THE PATHOGENESIS OF NAFLD

Diet is an important pathogenic factor of NAFLD. At present, it is well known that eating habits of patients with NAFLD/NASH differ greatly from a control group. In particular, patients with NAFLD/NASH consume food rich in saturated fats, cholesterol and fructose (soft drinks), and a diet poor in polyunsaturated fatty acids (fish and olive oil), fiber and antioxidants molecules such as vitamin C and vitamin E (19). An excessive consumption of high glycemic index food appears deleterious, as it favors hyperglycemia and hyperinsulinemia and stimulates de novo lipogenesis. In fact, high-glycemic index foods have been related to increased hepatic fat in both rodents and humans (20). Similarly, simple carbohydrates (fructose) stimulate hepatic de-novo lipogenesis and decrease lipid oxidation, thus leading to increased fat deposition. Conversely, monounsaturated lipids such as oleic acid (the primary component of olive oil), linoleic acid and n-3 fatty acids decrease the accumulation of intrahepatic lipids (21), (22), (23).
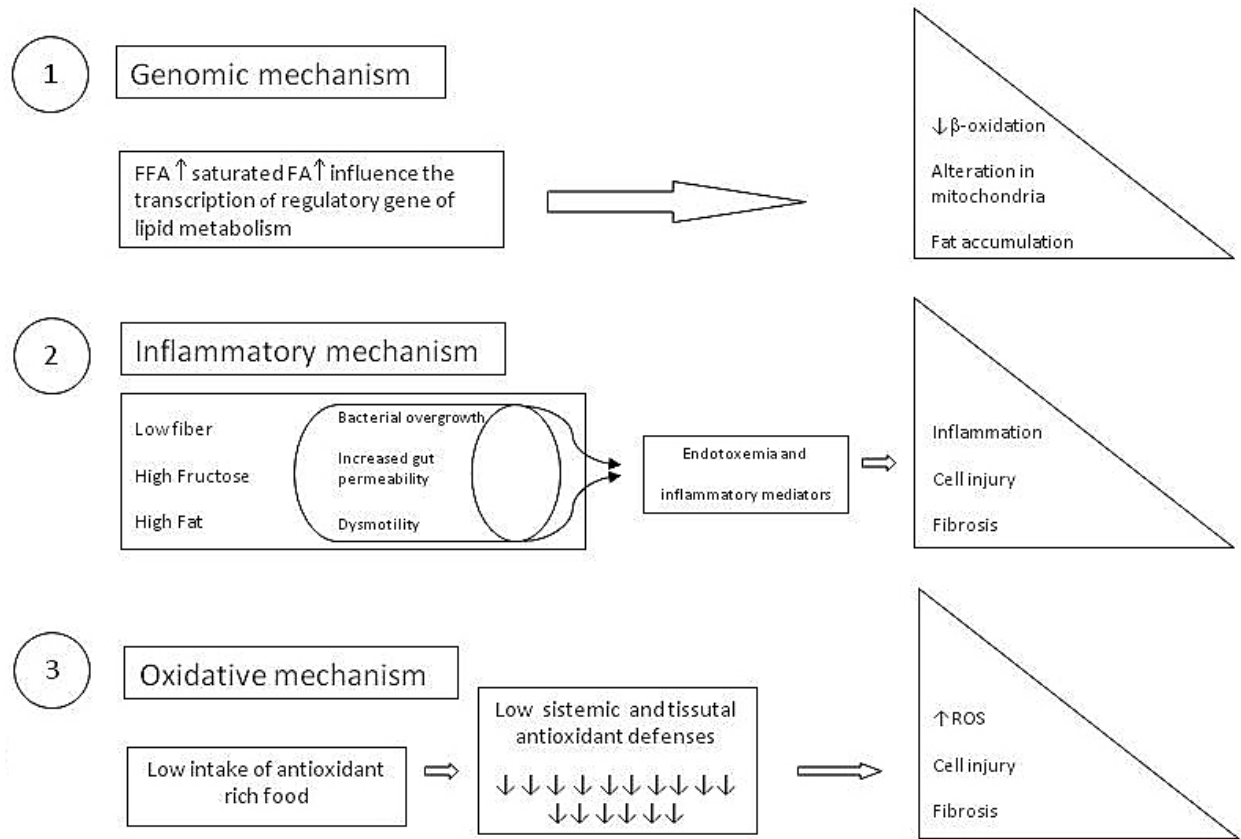
Figure 1. The role of food and its components in the pathogenesis of NAFLD.

Considering the pathogenic mechanism outlined above it is possible to envision at least 3 examples of how the diet, or the imbalance between the various food components can influence the emergence and progression of NAFLD (Figure1).

1. Genomic mechanism: It is well-known that the plasma level of some food components can influence the transcription of regulatory genes responsible for the activation/deactivation of enzyme systems of lipid metabolism. For example, polyunsaturated fatty acids and coffee are able to up-regulate the PPAR-α gene expression, favoring a higher rate of β-oxidation in the liver with a reduction of fat deposition (24), (25), (26).

2. Inflammatory mechanism: A diet deficient in fiber (with pre- and probiotic properties) can alter the intestinal flora and promote, (by modifying intestinal permeability), the translation of inflammatory cytokines through the portal vascular system in the liver. A diet rich in fruits, vegetables, omega 3 and coffee plays an anti-inflammatory role by inhibiting the secretion of specific pro-inflammatory cytokines (27), (24).

3. General oxidative mechanism: A diet low in antioxidant molecules (such as vitamin C and vitamin E, well represented in fruits and vegetables) reduces the total pool of antioxidant defenses and favors the oxidative damage (28).

5. THE APPROACH TO NAFLD WITH DRUGS

The increase in knowledge of the mechanisms that cause liver damage in NAFLD has stimulated the search for therapies that specifically target in contrasting the pathogenic mechanism of the disease (29).

The association of NAFLD with insulin resistance has provided the rationale for evaluating medical therapies that increase insulin sensitivity. The use of metformin is associated with normalisation of transaminases in 50% of cases, decreased steatosis assessed by ultrasonography, a partial improvement in necro-inflammation and less evident in fibrosis after 1 year of treatment (30). Although metformin was well tolerated and a biochemical improvement was shown, histological data remain very limited (31). Pioglitazone and Rosiglitazone are members of the thiazolidinedione family of antidiabetic agents that stimulate the peroxisome proliferator-activated receptor-gamma. They increase β-oxidation and reduce histological steatosis and inflammation, but unfortunately they do not show any influence on fibrosis (32) (33) (34) (35).

The depletion of antioxidant molecules within hepatocytes, resulting in impaired ROS inactivation, is the basis for antioxidant supplementation which is a potential treatment for NASH. The lipid –soluble antioxidant, α-tocoferol (vitamin E), has been demonstrated to inhibit lipid peroxidation and to suppress inflammatory cytokines such as tumor necrosis factor. Its use in the treatment of NASH improves steatosis, ballooning and inflammation (36).

Promising results are currently available for Silybin. In humans, Silybin acts as a free radical scavenger, which suppresses the proliferation of hepatic stellate cells and the deposit of collagen *in vitro*. Sylibin is rapidly absorbed in humans when conjugated with a phytosome and vitamin E, even if no comparison between histological data before and after treatment are available (37) (38).

Ursodeoxycholic acid (UDCA) is the most studied molecule for treatment of NAFLD, and initial pilot trials have proved to be promising as a NASH–specific therapy. UDCA could prevent steatosis by protecting against mitochondrial injury, by inducing a plasma membrane stabilizing effect, and by decreasing lipid peroxidation. Moreover, UDCA increases hepatocyte levels of glutathione and thio-containing proteins, therefore protecting hepatocytes against oxidative injury (39).

However, the only large controlled trial has shown that the use of UDCA has not documented any benefit in patients with NASH (40).

Beneficial effects of anti-TNF-alpha therapies have been demonstrated in animal models of NASH, and two pilot studies in patients with NAFLD have reported improvements in aminotransferase levels and histology. Given the emerging importance of pro-inflammatory cytokines in both liver pathology and insulin resistance, it seems likely that cytokines and their regulatory molecules will become major therapeutic targets in NAFLD in the near future (41).

Some types of antihypertensive drugs improve steatosis. In a study carried out by Georgescu *et al.* in 2009, valsartan and telmisartan, angiotensin receptor blockers with PPAR-g-modulating activity, was used in a randomized trial with 54 hypertensive NASH patients for 20 months. Both agents improved steatosis; telmisartan significantly improved ballooning, lobular inflammation and fibrosis compared to valsartan (42). Telmisartan significantly reduced insulin resistance, plasma tryglicerides, and total cholesterol whereas the blood pressure-lowering effects were similar to either agent. Currently, telmisartan is the only agent that improves fibrosis in NASH: whether the combination of PPARg and angiotensin receptor blockers activity of telmisartan may mediate its extensive metabolic and histological benefits still needs to be confirmed in larger RCTs (43).

The cannabinoid system has been increasingly emerging as a crucial mediator of acute and chronic liver injury. Recent experimental and clinical data have indicated that peripheral activation of cannabinoid CB1 receptors promotes insulin resistance and liver steatogenesis, two key steps in the pathogenesis of non-alcoholic fatty liver disease. Moreover, CB1 receptors activation have proved to enhance the progression of liver fibrogenesis. These findings provide a strong rationale for the use of CB1 antagonists in the management of NASH (44). Rimonabant, an antagonist of CB1 receptors, is able to reverse computed tomography-assessed steatosis and significantly decreased aminotrasferases (45), but it induced a series of neuropsychiatric effects that was deemed unacceptable by regulatory authorities, and both the drug and the trials were abruptly terminated (46).

The frequent coexistence of NAFLD with dyslipidemia and the increased cardiovascular risk of these patients make statins an attractive therapeutic tool in NAFLD. Despite these premises, data on statin efficacy in NAFLD are sparse due to feared hepatotoxicity of these drugs. A meta-analysis of 13 large trials have shown no increased risk of statin hepatotoxicity (47). Very recently, Harrison *et al.* found no beneficial effects of simvastatin treatment on biochemical and histological parameters in obese subjects with NASH (48).

Fibrates are safe and effective lipid-lowering agents in patients with NAFLD. Although this drug causes an improvement in liver tests and glucose levels, and decreases the percentage of subjects with metabolic syndrome, liver histology does not seem to improve significantly during treatment (49).

6. LIFE STILE MODIFICATION

There is consensus to declare that non-pharmacologic interventions aimed at correcting unhealthy lifestyles simultaneously treat all the clinical manifestations of the metabolic syndrome and are an effective treatment option in NAFLD (29).

Lifestyle changes include weight loss in overweight patients, dietary changes and increase of physical exercise (50).

As the disease stems from excess calorie intake and lack of physical activity, the correction of unhealthy lifestyles is the basis of any prevention and treatment strategy; drugs should remain a second-line treatment. Several studies have shown that the cornerstones of a healthy lifestyle: caloric restriction, weight loss and physical activity have a specific therapeutic role in NAFLD, improving insulin sensitivity, preventing disease progression and reducing the burden of the disease.

6.1. Weight loss

Despite the fact that weight loss and dietary and lifestyle changes are recommended as primary treatment for fatty liver, no specific guidelines exist pertaining the weight loss. The preponderance of the evidence supports the hypothesis that the moderate caloric restriction resulting in modest weight loss, (about 10% of initial weight), favourably modifies numerous surrogate end-points and is

Table 1. Effects of lifestyle modification in patients with NAFLD (Principal RTCs)

Title	Outcomes
Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E (53).	Low-fat diet and exercise were associated with improvement in liver enzymes, cholesterol, and plasma hyaluronic acid levels in patients with NASH.
Randomized controlled trial testing the effects of weight loss on non-alcoholic steatohepatitis (54)	Weight reduction achieved through lifestyle intervention leads to improvements in liver histology in NASH.
Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review (55).	Diet and increased physical activity induces weight loss and significant improvement in liver histology and laboratory abnormalities in pediatric NAFLD.
Effect of a 12-Month Intensive Lifestyle Intervention on Hepatic Steatosis in Adults With Type 2 Diabetes (56).	An intensive lifestyle intervention leading to 8% loss of body weight was successful in both reducing hepatic steatosis and decreasing the risk of incident NAFLD.

helpful in improving liver function tests and insulin sensitivity (51) but, to a lesser extent, histological endpoints. It is also suggested that overweight individuals and those with visceral obesity should follow a hypocaloric diet aimed at 0.5 kg/week weight loss (52). On the other hand, it is unknown whether these results can signify reduction in morbidity and mortality. The main papers published on the effectiveness of weight loss in patients with NAFLD are listed in Table 1.

6.2. Dietary approach

Dietary effects on whole-body metabolism and its regulation on hormones, transcription factors, and lipid metabolic pathways are considered to play a central role in NAFLD. It is now well documented that the increased prevalence of NAFLD and metabolic syndrome during the last decades was associated with deep modification of dietary habits, especially increased intakes of fats and simple carbohydrates.

In particular, the amount and type of fat intake can directly affect hepatic fatty infiltration and oxidative damage (57); and the energy consumption of fructose from sweetened beverages in patients with NAFLD was more high compared with control patients with non-steatotic livers ($p < 0.05$) (58).

Several studies have pointed out that the inclusion of n-3 and n-6 fatty acids, MUFAs, high contents of fiber and reduced intake of saturated fats, simple carbohydrates, and sweetened drinks may be universally recommended to NAFLD patients (59). On the other hand, very few studies on the effects of specific foods components on the features of NAFLD have been performed.

Preliminary results suggest that the consumption of n-3 fatty acids found in fish oils and walnuts is likely to improve blood lipid profiles and reduce inflammation, steatosis, and liver damage in NAFLD patients.

It has also been reported in literature that an olive oil-rich diet decreases accumulation of TGs in the liver, improves postprandial TGs, glucose and glucagonlike peptide-1 responses in insulin-resistant subjects, and upregulates glucose transporter-2 expression in the liver (60). The beneficial effects of olive oil against NAFLD were, principally, due to the decrease in NF-κB activation, a decrease in LDL oxidation and an improvement in insulin resistance.

The olive oil effects relate its mono-unsatured fatty acid composition and its polyphenols with anti-inflammatory and antioxidant properties. A preliminary study reported a significant improvement of liver echo-texture and of the Doppler Perfusion Index associated with a reduction of circulating liver enzymes, triglycerides and with a significant improvement of adiponectin levels after the long-term consumption of olive oil enriched with n-3 PUFA in patients with NAFLD (61). Furthermore, in a recent animal study Vitaglione *et al* reported that coffee consumption, and in particular the polyphenol component, protects the liver from damage caused by a high-fat diet in a rat model of steatohepatitis through the reduction of hepatic fat accumulation, systemic and liver endogenous antioxidant protection and liver inflammation (24).

Moreover, considering the ability of probiotics in inhibiting oxidative and inflammatory liver damage (62) and the capacity of tea extracts in reducing serum levels of liver enzymes, lipid peroxidation and production of mitochondrial reactive oxygen species, in animals models of NAFLD (63), healthy properties of probiotic and antioxidant-rich foods could also be investigated in NAFLD patients.

6.3. Physical activity

Prescriptive diets have a limited long-term efficacy; after a short period, most patients resume their old dietary habits and weight regain is the rule (64).

Physical activity, usually in combination with diet, but independent of weight loss, improves liver enzymes and reduces liver fat with uncertain results on hepatic necroinflammation (65).

Many studies have demonstrated that caloric restriction, weight loss and exercise training improve insulin sensitivity (66-69). These beneficial effects are now considered to reflect, at least in part, the effect of exercise on the activation of AMPK (5' adenosine monophosphate-activated protein kinase).

In addition, physical activity is able to decrease plasma insulin concentration by an increased hepatic clearance and by a reduced secretion mediated by a stimulation of alpha adrenergic receptors, which binds the

adrenalin level of the pancreatic beta cells in the insula. *In vitro* studies have shown that glucose uptake during muscle contraction occur through a mechanism independent of insulin and that insulin and muscle contraction have additive effects on glucose transport (70).

Finally, the addition of exercise training to a weight-loss program causes further improvement in other metabolic CHD (Coronary heart disease) risk factors and physical function. Therefore, diet therapy plus exercise is recommended to improve obesity- and aging-related derangements in metabolic and physical function in older adults (71).

Aerobic exercise and cardiorespiratory fitness are factors that may directly determine change in liver fat, beyond their effect on weight loss. In particular, cardiorespiratory fitness is closely associated with mitochondrial function, an important determinant of lipid oxidation (72).

However, there are no recognized criteria for the optimal intensity, duration, or total volume of exercise needed to obtain these beneficial effects. Physical training, consisting of 20 min cycling or running, 20 min swimming at submaximal heart rate, followed by 20 min of warm up/cool down three times per week for 4 weeks, resulted in a significant reduction in body weight and percentage of body fat, and this was associated with improved whole-body glucose uptake, decreased fasting insulin concentrations and increased circulating adiponectin and mRNA expression in muscle (73). However, very few studies have tested the effectiveness of intensive behavior therapy in NAFLD, aimed at lifestyle modifications to produce stable weight loss by reduced calorie intake and increased physical activity. On the other hand, recently, Kistler and colleagues showed that vigorous, but not moderate exercise, nor total duration or volume of physical activity, is related to decreased odds of having NASH or advanced fibrosis (74).

Prospective studies using the strict criteria to measure physical activity, addressing the role of concurrent weight loss, and assessing elementary histological features are needed to further clarify the effects of exercise on NAFLD.

On the other hand, most NAFLD patients fail to meet current recommendations for physical activity and it is very difficult to ensure that patients increase physical activity.

The behavioral approach may give patients the practical instruments to achieve their eating and exercise goals, incorporate them into a lifestyle and maintain the results for a long period, thereby possibly guaranteeing long-term durability of change. Cognitive-behavioral treatment should be provided to patients at risk of advanced liver disease, and this action should be coupled with prevention strategies at the population level. Only a synergistic approach and a global societal response might be effective in reducing the burden of advanced liver disease and premature death due to NAFLD/NASH (75).

7. FUNCTIONAL FOODS AND NAFLD

The concept of functional foods includes foods or food ingredients that exert a beneficial effect on host health and/or reduce the risk of chronic disease beyond their basic nutritional functions (76). The Institute of Medicine of the National Academy of Sciences (1994) has expanded this definition to ''any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains''. According to ILSI Europe functional foods are food products to be taken as part of the usual diet in order to have beneficial effects demonstrated scientifically to justify two specific type of claims: the enhanced function claim or the reduction of disease risk claim.

Interest in the health benefits of foods has been sparked by factors including rising health care costs (71), legislative changes that permit claims for foods and associated components (77) and by the emergence of new and exciting scientific discoveries (78).

The use of functional foods for the prevention and treatment of NAFLD has been poorly studied and up to day only few reports are encouraging and incite the promotion of functional food approach on NAFLD prevention on the basis of food and nutritional genomics (79) (80).

8. CONCLUSIONS

NAFLD is a rapidly emerging chronic liver disorder and will increasingly be a critical global health problem. Diet is an important pathogenic factor of NAFLD and there is consensus to declare that non-pharmacologic interventions aimed at correcting unhealthy lifestyles simultaneously are an effective treatment option in NAFLD. As the disease stems from excess calorie intake and lack of physical activity, the correction of unhealthy lifestyles is the basis of any prevention and treatment strategy while drugs should remain a second-line treatment.

All NAFLD patients should receive counselling for a low carbohydrate and low saturated fat diet, avoidance of fructose-enriched soft drinks and increased consumption of fruits and vegetables.

Currently, it is commonly accepted that the goal of reducing the number of people living with NAFLD could not be achieved without a global approach and the contributions of public health, special care and especially general practitioners.

9. REFERENCES

1. M Lazo, JM Clark: The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 28, 339-350(2008)

2. P Loria, LE Adinolfi, S Bellentani, E Bugianesi, A Grieco, S Fargion, A Gasbarrini, C Loguercio, A Lonardo, G Marchesini, F Marra, M Persico, D Prati, GS Baroni:

Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. *Dig Liver Dis* 42, 272-282 (2010)

3. LA Adams, JF Lymp, J St. Sauver, SO Sanderson, KD Lindor, A Feldstein, P Angulo : The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 129, 113–121 (2005)

4. LA Adams, KD Lindor : Nonalcoholic fatty liver disease. *Ann Epidemiol* 17, 863-869 (2007)

5. G. Bedogni, L Miglioli, F Masutti, C Tribelli, G Marchesini, S Bellentani: Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 42, 44–52 (2005)

6. CH Williams, J Stengel, MI AsikeI, DM Torres, J Shaw, M Contreras, CL Landt, SA Harrison: Prevalence of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis Among a Largely Middle-Aged Population Utilizing Ultrasound and Liver Biopsy: A Prospective Study. *Gastroenterology* 140, 124 –131 (2011)

7. E Hashimoto, K Tokushige: Prevalence, gender, ethnic variations, and prognosis of NASH. *Gastroenterology* 46, 63–69 (2011)

8. NJ Barshop, CS Francis, JB Schwimmer, JE Lavine: Nonalcoholic fatty liver disease as a comorbidity of childhood obesity. *Ped Health* 3, 271-281 (2009)

9. P Loria, A Lonardo, S Bellentani *et al*: Non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease: an open question. *Nutr Metab Cardiovasc Dis* 17, 684–698 (2007)

10. JG Fan, F Li, XB Cai, YD Peng, QH Ao, Y Gao: Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. *Gastroenterol Hepatol* 22, 1086-1091 (2007)

11.F Salamone F, Bugianesi E: Nonalcoholic fatty liver disease: The hepatic trigger of the metabolic syndrome. *Hepatology* 53, 1146-1147 (2010)

12. AD Burt, A Mutton, CP Day: Diagnosis and interpretation of steatosis and steatohepatitis. *Semin Diagn Pathol* 15, 246-258 (1998)

13. E Bugianesi, S Moscatiello, MF Ciaravella,G Marchesini: Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm Des* 16, 1941-1951 (2010)

14. SA Polyzos, J Kountouras, C Zavos: Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* 9, 299-314 (2009)

15.H Tilg, AR Moschen:.Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 52, 1836-1846 (2010)

16. V Paradis,P Bedossa: Definition and natural history of metabolic steatosis: histology and cellular aspects. *Diabetes Metab* 34, 638-642 (2008)

17. A Gastaldelli, M Kozakova,K Højlund, A Flyvbjerg, A Favuzzi, A Mitrakou, B Balkau:Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. *Hepatology* 49, 1537-1544 (2009)

18. A Gastaldelli, G Basta: Ectopic fat and cardiovascular disease: what is the link? *Nutr Metab Cardiovasc Dis* 20, 481-490 (2010)

19.W Nseir, F Nassar, N Assy : Soft drinks consumption and nonalcoholic fatty liver disease. *World J Gastroenterol* 16, 2579-2588 (2010)

20. M Zachut, A Arieli, H Lehrer, L Livshitz, S Yakoby, U Moallem: Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. *J Dairy Sci* 93, 5877-5889 (2010)

21. F Sofi, I Giangrandi, F Cesari, I Corsani, R Abbate, GF Gensini, A Casini:Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acid-enriched olive oil on non-alcoholic fatty liver disease patients: a preliminary study. *Int J Food Sci Nutr* 61, 792-802 (2010)

22. RM Machado, JT Stefano, CP Oliveira, ES Mello, FD Ferreira, VS Nunes, VM de Lima, EC Quintão, S Catanozi, ER Nakandakare, AM Lottenberg: Intake of trans fatty acids causes nonalcoholic steatohepatitis and reduces adipose tissue fat content. *J Nutr* 140, 1127-1132 (2010)

23. JP Allard, E Aghdassi, S Mohammed, M Raman, G Avand, BM Arendt, P Jalali, T Kandasamy, N Prayitno, M Sherman, M Guindi, DW Ma, JE Heathcote:Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol* 48, 300-307 (2008)

24. P Vitaglione, F Morisco, G Mazzone, DC Amoruso, MT Ribecco, A Romano, V Fogliano, N Caporaso, G D'Argenio: Coffee reduces liver damage in a rat model of steatohepatitis: the underlying mechanisms and the role of polyphenols and melanoidins. *Hepatology* 52, 1652-1661 (2010)

25. DB Jump: N-3 polyunsaturated fatty acid regulation of hepatic gene transcription.Curr Opin Lipidol 19, 242-247 (2008)

26. N Marx, H Duez, JC Fruchart, B Staels: Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circ Res* 94, 1168-1178 (2004)

27. H Yki-Järvinen: Nutritional modulation of nonalcoholic fatty liver disease and insulin resistance: human data. *Curr Opin Clin Nutr Metab Care* (2010)

28. N Assy, F Nassar, G Nasser, M Grosovski: Olive oil consumption and non-alcoholic fatty liver disease.*World J Gastroenterol* 21, 1809-1815 (2008)

29. S Bellentani, R Dalle Grave, A Suppini, G Marchesini: Behavior therapy for nonalcoholic fatty liver disease: the need for amultidisciplinary approach. *Hepatology* 47, 746–754 (2008)

30. MO Rakoski, AG Singal, MA Rogers, H Conjeevaram:Meta-analysis: insulin sensitizers for the treatment of non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 32, 1211-1221 (2010)

31. A Uygun, A Kadayifci, AT Isik, T Ozgurtas, S Devecis, A Tuzun, Z Yesilova, M Gulsen, K Dagalp: Metformin in the treatment of patients with non alcoholic steatohepatitis. *Aliment Pharmacol Threr* 26, 495–500 (2007)

32. PJ Hsiao, TJ Hsieh, KK Kuo, WW Hung, KB Tsai, CH Yang, ML Yu, SJ Shin: Pioglitazone retrieves hepatic antioxidant DNA repair in a mice model of high fat diet. *BMC Mol Biol* 26, 82 (2008)

33. LL Stein, MH Dong, R Loomba: Insulin sensitizers in nonalcoholic fatty liver disease and steatohepatitis: Current status. *Adv Ther* 26, 893-907 (2009)

34. GP Aithal, JA Thomas, PV Kaye, A Lawson, SD Ryder, I Spendlove, AS Austin, JG Freeman, L Morgan, J Webber: Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 135, 1176-84 (2008)

35. V Ratziu, P Giral, S Jacqueminet, F Charlotte, A Hartemann-Heurtier, L Serfaty, P Podevin, JM Lacorte, C Bernhardt, E Bruckert, A Grimaldi, T Poynard: LIDO Study Group Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. *Gastroenterology* 135, 100-110 (2008)

36. V Nobili, M Manco, R Devito, V Di Ciommo, D Comparcola, MR Sartorelli, F Piemonte, M Marcellini, P Angulo: Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. *Hepatology* 48, 119-128 (2008)

37. C Loguercio, A Federico, M Trappoliere, C Tuccillo, I De Sio, A DiLeva, M Niosi, MV D'Auria, R Capasso, C Del Vecchio Blanco: The effect of a Silybin-Vitamin E- Phospholipid complex on Nonalcoholc fatty liver disease: a pilot study. *Dig Dis Sci* 52, 2387–2395 (2007)

38. K Madan, Y Batra, DS Gupta, B Chander, KD Anand Rajan, R Singh, SK Panda, SK Acharya: Vitamin E-based therapy is effective in ameliorating transaminasemia in nonalcoholic fatty liver disease. *Indian J Gastroenterol* 24, 251-255 (2005)

39. MA Uzun, N Koksal, S Aktas, Y Gunerhan, H Kadioglu, N Dursun, AO Sehirli: The effect of ursodeoxycholic acid on liver regeneration after partial hepatectomy in rats with non-alcoholic fatty liver disease. *Hepatol Res* 39, 814-821 (2009)

40. R Orlando, L Azzalini, S Orando, F Lirussi: Bile acids for non-alcoholic fatty liver disease and/or steatohepatitis. *Cochrane Database Syst Rev* (2007)

41. J Poniachik, A Csendes, JC Díaz, J Rojas, P Burdiles, F Maluenda, G Smok, R Rodrigo,LA Videla: Increased production of IL-1alpha and TNF-alpha in lipopolysaccharide-stimulated blood from obese patients with non-alcoholic fatty liver disease. *Cytokine* 33, 252-257 (2006)

42. EF Georgescu, R Ionescu, M Niculescu, L Mogoanta, L Vancica: Angiotensin-receptor blockers as therapy for mild-to-moderate hypertension-associated non-alcoholic steatohepatitis. *World J Gastroenterol* 15, 942-954 (2009)

43. G Musso, R Gambino, M Cassader, G Pagano: A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 52, 79-104 (2010)

44. A Mallat, S Lotersztajn: Cannabinoid receptors as novel therapeutic targets for the management of non-alcoholic steatohepatitis. *Diabetes Metab* 34, 680-684 (2008)

45. JP Després, R Ross, G Boka, N Alméras, I Lemieux: Effect of Rimonabant on the high triglycerides/low HDL cholesterol dyslipidemia, intraabdominal adiposity and liver fat; The ADAGIO lipids trial. *Arterioscler Thromb Vasc Bio* 29, 416-423 (2009)

46. DL Kelly, DA Gorelick, RR Conley, DL Boggs, J Linthicum, F Liu, S Feldman, MP Ball, HJ Wehring, RP McMahon, MA Huestis, SJ Heishman, KR Warren, RW Buchanan: Effects of the cannabinoid-1 receptor antagonist rimonabant on psychiatric symptoms in overweight people with schizophrenia: a randomized, double-blind, pilot study. *J Clin Psychopharmacol* 31, 86-91 (2011)

47. S de Denus, SA Spinler, K Miller, AM Peterson: Statins and liver toxicity: a meta-analysis. *Pharmacotherapy* 24, 584-591 (2004)

48. A Nelson, DM Torres, AE Morgan, C Fincke, SA Harrison: A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: a randomized placebo-controlled trial. *J Clin Gastroenterol* 43, 990–994 (2009

49. C Fernández-Miranda, M Pérez-Carreras, F Colina, G López-Alonso, C Vargas, JA Solís-Herruzo: A pilot trial of fenofibrate for the treatment of non-alcoholic fatty liver disease. *Dig Liver Dis* 40, 200-205 (2008)

50. D Vancampfort, M Probst, K Sweers, K Maurissen, J Knapen, M De Hert: Relationships between obesity, functional exercise capacity, physical activity participation

and physical self-perception in people with schizophrenia. *Acta Psychiatr Scand* (2011)

51. B Lam, ZM Younossi : Treatment options for nonalcoholic fatty liver disease. *Therap Adv Gastroenterol* 3, 121-37 (2010)

52. C Gasteyger, TM Larsen, F Vercruysse F *et al*:. Effect of a dietary-induced weight loss on liver enzymes in obese subjects. *Am J Clin Nutr* 87, 1141–7 (2008)

53. M Kugelmas, DB Hill, B Vivian, L Marsano, CJ McClain: Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology* 38, 413-419 (2003)

54. K Promrat, DE Kleiner, HM Niemeier, E Jackvony, M Kearns, JR Wands, JL Fava, RR Wing: Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 51, 121-129 (2010)

55. V Nobili, A Alisi, N Panera, C Agostoni: Low birth weight and catch-up-growth associated with metabolic syndrome: a ten year systematic review. *Pediatr Endocrinol Rev* 6, 241-247 (2008)

56. M Lazo, SF Solga, A Horska, S Bonekamp, AM Diehl, FL Brancati, LE Wagenknecht, FX Pi-Sunyer, SE Kahn, JM Clark: Fatty Liver Subgroup of the Look AHEAD Research Group Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care* 33, 2156-2163 (2010)

57. G Musso, R Gambino, F De Michieli, M Cassader, M Rizzetto, M Durazzo, E Fagà, B Silli, G Pagano: Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 37, 909-916 (2003)

58. X Ouyang, P Cirillo, Y Sautin, S McCall, JL Bruchette, AM Diehl, RJ Johnson, MF Abdelmalek: Fructose consumption as a risk factor for non-alcoholic fatty liver disease. *J Hepatol* 48, 993-999 (2008)

59. AM Zivkovic, JB German, AJ Sanyal: Comparative review of diets for the metabolic syndrome:implications for nonalcoholic fatty liver disease. *Am J Clin Nutr* 86,285–300 (2007)

60. N Assy, F Nassar, G Nasser, M Grosovski: Olive oil consumption and non-alcoholic fatty liver disease. *World J Gastroenterol* 21, 1809-1815 (2009)

61. F Sofi, I Giangrandi, F Cesari, I Corsani, R Abbate, GF Gensini, A Casini: Effects of a 1-year dietary intervention with n-3 polyunsaturated fatty acid-enriched olive oil on non-alcoholic fatty liver disease patients: a preliminary study. *Int J Food Sci Nutr* 61, 792-802 (2010)

62. E Esposito, A Iacono, G Bianco, G Autore, S Cuzzocrea, P Vajro, R Berni Canani, A Calignano, G Mattace Raso, R Meli: Probiotics Reduce the Inflammatory Response Induced by a High-Fat Diet in the Liver of Young Rats . *J Nutr* 139, 905–911 (2009)

63. K Nakamoto, F Takayama, M Mankura, Y Hidaka, T Egashira, T Ogino, H Kawasaki, A Mori: Beneficial Effects of Fermented Green Tea Extract in a Rat Model of Non-alcoholic Steatohepatitis. *J Clin. Biochem Nutr* 44, 239–246 (2009)

64. NA Johnson, J George: Fitness versus fatness: moving beyond weight loss in nonalcoholic fatty liver disease. *Hepatology* 52, 370-381 (2010)

65. J Frith, CP Day, L Robinson, C Elliott, DE Jones, JL Newton: Potential strategies to improve uptake of exercise interventions in non-alcoholic fatty liver disease. *J Hepatol* 52, :112-116 (2010)

66. BH Goodpaster, A Katsiaras, DE Kelley: Enhanced fat oxidation through physical activity is associated with improvements in insulin sensitivity in obesity. *Diabetes* 52, 2191–2197 (2003)

67. DR Dengel,Re Pratley, JM Hagberg, EM Rogus, AP Goldberg:. Distinct effects of aerobic exercise training and weight loss on glucose homeostasis in obese sedentary men. *J Appl Physiol* 81, 318–325 (1996)

68. R Ross, D Dagnone, PHJ Jones, H Smith, A Paddags, R Hudson, I Janssen: Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med* 133,92–103 (2000)

69. A Franssila-Kallunki, A Rissanen, A Ekstrand, A Ollus, L Groop: Weight loss by very-low-calorie diets: effects on substrate oxidation, energy expenditure, and insulin sensitivity in obese subjects. *Am J Clin Nutr* 56, 247–248 (1992)

70. R Kelishadi, SR Cook, B Amra, A Adibi: Factors associated with insulin resistance and non-alcoholic fatty liver disease among youths. *Atherosclerosis* 204, 538-543 (2009)

71. K Shah, A Stufflebam, TN Hilton, DR Sinacore, S Klein, DT Villareal: Diet and Exercise Interventions Reduce Intrahepatic Fat Content and Improve Insulin Sensitivity in Obese Older Adults. *Obesity* (Silver Spring) 17, 2162–2168 (2009)

72. K Kantartzis, C Thamer, A Peter, J Machann, F Schick, C Schraml, A Königsrainer, I Königsrainer, S Kröber, A Niess, A Fritsche, HU Häring, N Stefan: High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut* 58, 1281-1288 (2009)

73. M Bluher, J W Bullen, J H Lee *et al*: Circulating adiponectin and expression of adiponectin receptors in human skeletal muscle: associations with metabolic

parameters and insulin resistance and regulation by exercise. *J Clin Endocrinol Metab* 91, 2310–2316 (2006)

74. KD Kistler, EM Brunt, JM Clark, AM Diehl, JF Sallis, JB Schwimmer: Physical activity recommendations, exercise intensity, and histological severity of nonalcoholic Fatty liver disease. *Am J Gastroenterol* 106, 460-468 (2011)

75. E Centis, R Marzocchi, S Di Domizio, MF Ciaravella, G Marchesini: The effect of lifestyle changes in non-alcoholic fatty liver disease. *Dig Dis* 28, 267-273 (2010)

76. AC Huggett, B Schliter: Research needs for establishing the safety of functional foods. Nutr. Rev 54, S143– S148 (1996)

77. FM Clydesdale: A proposal for the establishment of scientific criteria for health claims for functional foods. *Nutr. Rev* 55, 413–422 (1997)

78. MK Kottke: Scientific and regulatory aspects of nutraceutical products in the United States. *Drug Dev In. Pharm* 24, 1177–1195 (1998)

79. D Farr: Functional foods. *Cancer Lett* 114, 59–63 (1997)

80. EJ Kim, E Kim, EY Kwon, HS Jang, CG Hur, MS Choi:.Network analysis of hepatic genes responded to high-fat diet in C57BL/6J mice: nutrigenomics data mining from recent research findings. *J Med Food* 13, 743-756 (2010)