Dietary lipids and NAFLD : suggestions for improved nutrition

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Abstract

Non-alcoholic fatty liver disease (NAFLD) ranges from steatosis and hepatic insulin resistance to non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis. NAFLD is now considered as the hepatic manifestation of the metabolic syndrome, and both are triggered by mechanisms including inflammation, lipid overload and oxidative stress in adipose tissue and liver. Despite accumulation of numerous data on NAFLD physiopathology, therapeutic modulation of the pathways involved appear insufficiently efficient or associated with serious adverse effects. The increased prevalence of NAFLD and metabolic syndrome during the last decades was associated with deep modifications of dietary habits, especially increased fat intakes. Recent literature provides clues of increased saturated (SFA) and n-6 polyunsaturated fatty acids (PUFA) as well as reduced n-3 PUFA in the diet of NAFLD and NASH patients. Indeed, strong data support the detrimental role of high SFA and n-6/n-3 ratio as well as low monounsaturated fatty acids (MUFA) and n-3 PUFA on metabolic parameters, which are ameliorated by administration of n-3 PUFA and MUFA. Despite governments and health associations having revised their recommendations for n-3 PUFA intakes upward during the last decade, those are still inferior to levels proved of therapeutic efficiency and are still not reached in the general population. This short review discusses these issues and provides consequent pragmatic suggestions for enhanced dietary measures for prevention of NAFLD and metabolic syndrome in the general population. (Acta gastroenterol. belg., **2010**, 73, **431-436**).

Key words : NAFLD, dietary recommendations, saturated fatty acids, monounsaturated fatty fatty acids, poyunsaturated fatty acid.

Abbreviation list

AA : arachidonic acid ; ALA : alpha-linolenic acid ; DHA : docosahexaenoic acid ; EPA : eicosapentaenoic acid ; ER : endoplasmic reticulum ; FA : fatty acids, IR : insulin resistance ; LC : long chain ; MetS : metabolic syndrome ; MUFA : monounsaturated fatty acids ; NAFLD : non-alcoholic fatty liver disease ; NASH : non-alcoholic steatohepatitis ; PUFA : polyunsaturated fatty acids ; SFA : saturated fatty acids.

Introduction

The metabolic syndrome (MetS) is defined as a cluster of symptoms such as visceral obesity, insulin resistance, elevated blood pressure and dyslipidemia, associated with increased risk of type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver diseases (NAFLD), and some types of cancers (1,2). This pathological condition is currently reaching epidemic proportions and may soon represent the first health issue worldwide in terms of health-care community cost and mortality, even in developing countries. Although multifactorial processes participating are yet to be unraveled, there is a general agreement that the MetS is largely subtended by low grade and systemic inflammatory conditions (3), with an imbalance between pro- and anti-inflammatory molecules and elevated serum markers of inflammation (4). Increased macrophage infiltration in adipose tissue (5) as well as recruitment of lymphocytes (6) are recognized causes of inflammation and insulin resistance in this context. Recently, experimental data strongly support that liver inflammation, and in particular activation/recruitment of Kupffer cells, directly participates in diet-induced insulin resistance (7,8). As the insulin-resistant liver largely accounts for hyperglycaemia and is also a source of cardiovascular risk factors (9,10), the implication of the liver compartment is an absolute requirement both for the cardiovascular risk and for progression towards type 2 diabetes.

Non-alcoholic fatty liver diseases

NAFLD comprise a disease spectrum ranging from simple steatosis and hepatic insulin resistance to non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis (10,11). NAFLD occurrence undoubtedly correlates with the presence of symptoms of the MetS and is now considered as its hepatic expression (1,10). While NAFLD affects 10-35% of adult populations worldwide, its prevalence increases up to 80-90% in obese and hyperlipidaemic patients (12). To the same extent, NAFLD is seen in 3-10% of children, but in up to 40-70% of obese children. Taken together, these data point out that NAFLD prevalence, as associated to obesity and MetS, may follow a similar rise and become the more prevalent liver disease and a major public health burden in the years to come (12-14).

Cellular and molecular mechanisms

The major events in the pathogenesis of NAFLD were primarily schematized by the “two hits” hypothesis, with steatosis and insulin resistance thought to occur first in

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Submission date : 14/10/2010
Acceptance date : 23/10/2010

the context of unbalanced diets or MetS, followed by mitochondrial dysfunctions and oxidative stress, that trigger inflammation and fibrogenesis in the primed liver (15). Studies conducted since have suggested that the causal and sequential links between these alterations were not as clarified as supposed and that this model should be revised in a more dynamic light (1,11). Liver fat accumulation may represent a protective rather than a noxious mechanism and therefore NAFLD/NASH may be the consequence of failure of such protective process (1,16). Hepatic insulin resistance (IR) and inflammation may result from the exposure of the (fatty) liver to oxidative stress (17) or metabolic and inflammatory mediators produced by the adipose tissue (18). There is also a possibility that dietary factors or steatosis itself could induce a low-grade inflammatory response and insulin resistance in the liver (19,20). Indeed, lipid load in hepatocytes results in increased generation of hepatotoxic free oxygen radicals and lipid peroxides from beta-oxidation, which induce mitochondrial dysfunctions and endoplasmic reticulum (ER) stress, all of which trigger inflammation (1). Interestingly, early Kupffer cell activation during high-fat diet without obvious signs of adipose tissue inflammation seems sufficient to induce hepatic IR in mice (8). This, together with other reports, supports a more central role of liver inflammation in MetS and NAFLD. With time, chronic ER, oxidative stress and inflammation trigger cellular damage and replacement that pave the way for fibrosis and evolution towards NASH, cirrhosis, and HCC (11).

Various processes and signaling pathways implicated in NAFLD/NASH pathogenesis are extensively reviewed eslewhere and include : oxidative stress ; cytokines and inflammatory chemokines such as adiponectine, TNFa, IL6, and NO ; nuclear receptors PPARs, RXR and LXR ; transcription factor as SREBPs, NFκB and JNK ; and lipid-derived mediators such as endocanabinoids and eicosanoids (11,21,22). Despite intensive research conducted in this field, effective NAFLD/NASH treatment is currently lacking and focuses on amelioration of risk factors (23). On the one hand, lifestyle therapy, although efficient both on MetS and NAFLD/NASH, is hard to maintain in the long term (24). On the other hand, chemical modulations of the pathways mentioned above appear insufficiently efficient or associated with serious adverse effects, as rimonabant (23,25). Thus, while awaiting pharmaceutical options, it is time for pragmatic reconsideration of the prime role of nutrition in NAFLD.

Influence of nutritional behaviour

Contemporary to the rise in MetS prevalence, important changes in human nutrition and dietary habits were observed, with increased consumption of intensively produced- and processed-food. The latter are usually rich in fats and refined carbohydrates and poor in essentiel nutrients such as anti-oxidants, fibers, and n-3 polyunsaturated fatty acids (PUFA). In particular, fatty acids (FA) consumption has increased significantly such as, in today's diet, FA represent 28-42% of total energy consumed by European populations (26), which is at least one third more than in 1961 (27). Also, qualitative changes in dietary FA have occurred over the past 50 years with increased consumption of saturated fat especially from meat and dairy products. Diet also evolved towards higher intakes in vegetable oils rich in n-6 PUFA and poor in n-3 PUFA (such as safflower) and relative decrease in n-3 PUFA intakes (28) related to insufficient consumption of fatty fish, nuts, seeds, whole-grain cereals (29). As a result, n-6 PUFA consumption has become progressively much higher than that of n-3 PUFA (30), so that Western diets have a n-6/n-3 ratio ranging from 10/1 to 20/1 for a ratio of 1/1 in the diet of our ancestors.

During the last decade, many studies conducted independently around the world have provided convincing evidence of the detrimental role of increased saturated FA (SFA), trans-fats, cholesterol and refined sugar (in particular high fructose corn sirup) as well as of low n-3 PUFA, fibers, and anti-oxidants dietary intakes on the MetS and the cardiovascular risk (29,31,32). In 2007, Zelber-Sagi *et al.* assessed food consumption of 108 patients with NAFLD using semi-quantitative food-frequency questionnaire (33). Those authors revealed that patients with NAFLD consume twice the quantity of soft drinks and eat more meat (27%) compared with the "normal liver" group, and that these dietary differences are associated with an increased risk of NAFLD independently of traditional risk factors. Patients with NAFLD also tended to consume less oily fish (20-25%), but the difference did not reach statistical significance ($p = 0.056$). This was the first convincing evidence of an association between nutritional pattern and NAFLD. In the same way, patients with NASH were reported to absorb more saturated as well as n-6 polyunsaturated FA and less n-3 PUFA than healthy individuals (34).

Relevant to NAFLD/NASH pathogenesis, and irrespective of dietary habits, fatty liver and steatohepatitis have been associated with alterations in the bioavailability of long-chain (LC) PUFA - namely eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic (AA) acids – as well as with a significant decrease in hepatic LC PUFA independent of the level of their precursors – alpha-linolenic acid (ALA) for EPA/DHA and linoleic acid (LA) for AA – (35,36). Furthermore, both high hepatic n-6/n-3 ratio and decreased EPA stores in liver correlated significantly with the quantity of hepatic triglycerides in NAFLD patients (37) so that any imbalances in n-6/n-3 ratio as well as low n-3 PUFA levels were proposed as factors inducing or sustaining metabolic-inflammatory pathologies such as NAFLD. As LC n-3 PUFA are mostly produced in the liver under physiological conditions, alterations of their metabolism during NAFLD could be both a consequence of reduced n-3 PUFA intakes and a cause of reduced n-3 LC PUFA bioavailability.

Thus, despite the lack of repeated cohort studies clearly disentangling dietary influence on NAFLD, indications that altering dietary FA composition could possibly modulate NAFLD are plentiful.

Saturated fatty acids

A body of epidemiological data highlights that high consumption of SFA has adverse effects on lipid and glucose homeostasis and evolution towards the MetS (38) and, hence, NAFLD. Interestingly, the pathways affected by SFA overlap with those implicated in NAFLD/NASH (39). Recent experiments conducted in mice exposed to different isocaloric diets rich in saturated fats demonstrated that the deterioration of hepatic insulin sensitivity is partly dependant on the presence of stearate (40). Considering that the main nutritional source of stearate is animal fat, these results strengthen the implication of unbalanced diet in NAFLD development, especially the increased meat ingestion noticed in patients with fatty liver (33). However, strict management of dietary reduction of total or specific SFA is difficult to achieve in practice, and strong data on the therapeutic effects of such reduction on NAFLD are thus still needed.

Mono-unsaturated fatty acids

Many epidemiological studies report the health, inflammatory, and cardiovascular benefits of the mediterranean diet, which is rich in monounsaturated fatty acids (MUFA) (41). However, this influence is not clearly attributable to MUFA as this diet is also rich in fish, vegetables, fruits, and grains, which are important sources of n-3 PUFA (see below) and anti-oxidants. In 2007, a rat experiment demonstrated that olive oil alone (73% MUFA) reduced steatosis by 30% in the context of methionine-choline deficient diet, probably due to inhibition of hepatic triacylglycerol synthesis and stimulation of hepatic peroxisomal beta-oxidation (42).

In clinical trials, increased MUFAs led to a reduction in glucose, blood pressure, VLDL, and chylomicron remnants as well as to an increase in HDL in patients with type 2 diabetes mellitus (43,44). In insulin-resistant subjects, olive oil-rich diet was shown to improve postprandial triglycerides and glucose levels, which are dependant on hepatic metabolism, and to increase glucose transporter 2 in the liver as well as insulin sensitivity (45). Beside MUFA, unrefined or virgin olive oil also contains bioactive compounds, such as polyphenols and squalene, with intrinsic anti-inflammatory and antioxidant properties.

Therefore, an increase in MUFA intakes, particularly as a replacement for SFA, may both offset the pro-inflammatory effects of SFA and favor decreased insulin resistance and steatosis, notably through modulation of FA metabolism. Further studies in humans are still needed to ascertain whether the consumption of MUFA, which most accessible source in occident are olive and rapeseeds oils, and naturally associated micronutrients may be helpful in reducing NAFLD parameters.

Poly-unsaturated fatty acids

PUFA of the n-3 and n-6 series are termed "essential" as animals cells are not equipied for their biosynthesis but are necessary for membranes fonctionality and lipid-derived mediators production. Thus, they must be provided by diet. The n-3 PUFA series comprise the essential ALA, found in wallnut, soy and rapeseed oils, which serves as precursor for long chain (LC) n-3 PUFA, EPA and DHA, almost exclusively found in marine fish. The n-6 series of PUFA is composed of the essential precursor LA, and its long chain derivate AA, both of which are found in high quantities in sunflower, grapeseed, and corn oils but in small quantities in many foodstuffs from both animal and vegetable reigns. Precursors of both n-3 and n-6 series of PUFA are in competition for enzymatic processing by D5- and D6-desaturases to their respective LC metabolites EPA/DHA and AA, precursors for anti-inflammatory eicosanoids/autacoids and pro-inflammatory eicosanoids synthesis by cycloxygenases and lipoxygenases (46-48). As the two series are competitive substrates for the same processing enzymes, the production of pro/anti-inflammatory metabolites is influenced by both absolute and relative amounts of n-6 and n-3 PUFA (n-6/n-3 ratio).

EPA and DHA are the most biologically active n-3 PUFA and exhibit increasingly documented protective effects. Those converge to reduction of low grade inflammation and vascular lesions and shift from lipogenesis to lipolysis through several mechanisms reviewed elsewhere (47-52). Of specific interest, in the liver, EPA/DHA stimulate β-oxidation and insulin sensitivity and inhibit lipogenesis and inflammation by activation of transcription factors controlling lipolysis (as PPAR-α and LXR), and inhibition of lipogenic (ChREBP, SREBP-1c) and pro-inflammatory (AP-1 and NF-κB) transcription factors (25,49), as well as by yielding anti-inflammatory eicosanoids (48) and autacoids (47,48,50). Thus, considering that the conversion rate of ALA to EPA/DHA is low in humans, exogenous sources of EPA/DHA must be present in the diet to ensure physiological protective cellular levels of n-3 PUFA.

Consistently with these effects, literature provides a profusion of data reporting that increased n-3 PUFA consumption in intervention studies alleviates MetS (38, 46,53,54), dyslipidaemia (55), cardiovascular diseases (38,56) and some cancers (57). n-3 PUFA may also improve glucose homeostasis and insulin sensitivity, but this remains to be confirmed in type 2 diabetes mellitus (30,58). Data obtained from animals suggested that n-3 PUFA could represent a potential treatment for NAFLD (1). Consistently, three recent studies reported that n-3 PUFA administration (1-2.7 g/d for 6-12 months) to patients with characterized NAFLD improved biochemical, ultrasonographic and haemodynamic features

of liver steatosis, inflammation and fibrosis (59-61). Those observations should encourage larger controlled clinical trials with liver biopsy as an end point to properly assess the therapeutic potential effects of n-3 PUFA on NAFLD.

Opposite to n-3 PUFA effects, excessive n-6 PUFA or high n-6/n-3 ratio in diet and/or tissues were associated with worsen inflammatory (37,62), metabolic (62,63), and cardiovascular parameters as well as risk of NAFLD (35,37,63). These deleterious effects are notably attributable to the competition between n-6 and n-3 PUFA for desaturases binding and ensuing signaling molecules production. Indeed, excessive n-6 PUFA and high n-6/n-3 ratio result in displacement of PUFA metabolism balance towards increased production of n-6 derived pro-inflammatory eicosanoids at the expense of n-3 anti-inflammatory derivates.

Beside nutrition, acquired modifications in enzymatic machinery metabolizing PUFA have been described in association with obesity, insulin resistance and cancer (49,64). As expression and activity of desaturases are modulated by insulin, LC-PUFA, oxidative stress and transcription factors (PPAR alpha and SREBP1) (25), the MetS state itself influences on LC-PUFA metabolism. Indeed, a recent study based on measurement of direct enzymatic activity in liver biopsies demonstrated defective desaturation activity in NAFLD obese patients (65), which was further correlated with insulin resistance. It is also of note that lipoxigenase-derived AA metabolites are increased in the liver from patients with NAFLD and NASH, and that it may influence NAFLD/NASH pathogenesis (66). Thus, NAFLD may be both a consequence and a cause of defective hepatic PUFA metabolism.

Towards a nutritional managment of NAFLD ?

The studies reviewed above provide reliable clues for the detrimental role of unbalanced FA intakes, notably diets excessive in FA, SFA and n-6 PUFA, and poor in MUFA and n-3 PUFA, on liver physiology. Conversely, diets rich in MUFA as well as administration of n-3 PUFA improve NAFLD risk-factors. To our opinion, these unsaturated FA must therefore be considered as safe and cost-effective tools for preventive and therapeutical management of NAFLD and MetS (59-61).

Currently, governments (France, Belgium, UK, Netherlands, New Zealand, Australia) and health organizations (FAO/WHO, American dietetic association, American heart association) globally recommend that FA represent around 20-35% (80-90 g) of total energy supplies, from which 1.4 to 2.5 g/d of total n-3 PUFA, with a n-6/n-3 ratio between 4-5 and an EPA/DHA intake of 500 mg/d (ranging from 140 to 600 mg/d depending on the authority issuing guidelines) (67-69). Two servings of fish per week (30-40 g/d), one of which oily (salmon, tuna, mackerel, sardine), represent a strict minimum for fulfillment of such LC n-3 PUFA inputs.

These recommendations are far from being followed by the general population (30,70) and difficult to reach in institutions in charge of catering (71). For daily enhancement of the "lipidic" management of NAFLD and MetS, we would like to share three suggestions easy to implement :

First, sources of saturated fats should be avoided in nutrition much as possible, especially foodstuffs with poor nutritional values, such as butter fat and industrialized food, as well as meat rich in lipids (as pork, lard, sausages, fatty hash meat, chicken skin...). Such a change in dietary habits would intrinsically also reduce total FA content of the diet.

Second, olive oil (71% MUFA) should be preferred to processed and safflower-derived oils (rich in either SFA or n-6 PUFA) often used for cooking, especially as MUFA are less suseptible than PUFA to heat-induced production of deleterious oxidation products (72).

Third, LC n-3 PUFA amounts administered in interventional studies reporting a beneficial effect on MetS and NAFLD were above the current recommendations (140-600 mg/d) and ranged from 1 to 9 g/d, without any side effects (59-61,67). Ancestral nutrition, to which our metabolism is believed to be best fit, provided around 5-6 g/d of total n-3 PUFA (73). Thus, dietary recommendation aiming at both maximizing the protective influence of n-3 PUFA for the general population and taking into account interindividual variability, should correspond to such minimal reported curative level of 1 g/d of EPA/DHA (4 servings/week of fish, half oily) within a high total intake of n-3 PUFA and a n-6/n-3 ratio ideally reaching those of our ancestors. To this end, fish, nuts, seeds as well as grains consumption should be promoted and dressing oils, which are commonly prepared with safflower oil, should be half, if not totally, replaced by oils rich in n-3 PUFA (rapeseed, wallnut, soy), which contain sufficient levels of n-6 PUFA for fulfillment of recommendations.

Those are unsophisticated modifications that could be readily implemented, providing proper education to a modified taste of the food by oils rich in beneficient FA (MUFA/n-3 PUFA). In this respect, both dieticians and practitioners have an important role to play. Interestingly, we recently had the opportunity to consider the second and third suggestions in collaboration with dieticians in charge of school catering (71). This led to a valuable enhancement of FA balance, with a surprisingly spontaneous acceptance by children as observed by the staff.

Acknowledgements

This work was supported by FRS-FNRS grants (N°3.4578.07 and M.IS : F.45.08.08), la Direction de la Recherche Scientifique de la Communauté Française de Belgique (ARC grant, 05/10-328), University FSR grants to I.L. ; O.M.C. and I.L. are FRS-FNRS post-doctoral researcher and reseach associated, respectively.

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