Experimental observations and clinical implications of fasting and diet supplementation in fatty livers


Abstract. – Fatty accumulation per se does not appear to affect liver function; however, interest has recently renewed to fatty liver because of the clinical relevance of non alcoholic steato-hepatitis (NASH) and for the increased risk of post-transplant failure in grafted livers with steatosis.

Clinical and experimental studies have doubtless demonstrated that oxidative stress ensues in steatotic livers.

Mitochondria represent the preferential target of the oxidative injury associated to fatty degeneration and show reduced content of glutathione, higher levels of oxidative products and damages to enzymes involved in the process of ATP synthesis, which become more evident under stressing conditions.

Although obese patients with fatty liver are advantaged by weight loss, clinical and experimental observations suggest that fatty livers poorly tolerate excessive food deprivation. These observations represent the rationale for treatment strategies based on the supplementation of antioxidants and energetic substrates rather than solely a diet restriction.

This review focuses on data emerging from a series of investigations performed in rats with fatty livers induced by a choline-deficient diet, which resembles human steatosis due to an excessive intake of carbohydrates, and aims to give the cue for the development of therapeutic options able to preserve hepatic function after transplantation of steatotic organs.

Key Words: Fasting, Fatty liver, Non alcoholic steatohepatitis, Oxidative stress.

Introduction

Fatty degeneration of the liver identifies a liver in which lipid, mainly triglyceride, accounts for more than 5% of the whole organ weight and represents the most common liver alteration in the general population. Fatty accumulation in hepatocytes, primary steatosis, follows to a variety of common conditions including obesity, malnutrition, malabsorption, diabetes, dysthyroidism or is secondary to alcohol abuse, drug intake, hepatitis C infection, iron or copper accumulation. Fat accumulates in parenchymal liver cells as a result of excessive free fatty acids delivery to the liver, an increased mitochondrial synthesis of fatty acids, or the failure of the synthesis and secretion of triglycerides and/or apolipoproteins.

Because fatty accumulation per se does not appear to impair liver function, steatosis was considered a benign condition with low risk of evolution. Recently, however, the interest in the liver steatosis has been growing due to the increasing number of liver transplants, which greatly exceeds the number of available organs, and the need of healthy livers. In fact, grafted livers with steatosis, accounting for about 30% of the donor pool, are at increased risk of post-transplant failure and are actually discarded for donation. Therefore, a better understanding of the mechanisms underlying fatty degeneration of hepatocytes and the high rate of post-transplant complications might lead to the development of therapeutic strategies able to preserve hepatic function after transplantation.
The term NASH (non alcoholic steato-hepatitis) indicates an increasingly recognised condition with possible progression toward end-stage liver disease. Although the histology resembles that of alcoholic liver injury, the disease occurs in patients with minor or even absent alcohol intake. Once other causes are excluded, NASH is confirmed by liver biopsy and represents a common explanation for asymptomatic elevation of aminotransferases in 40-90% of cases. The primary metabolic abnormality switching fatty livers to NASH is still unknown; insulin resistance may influence several intracellular mechanisms and is associated with increased cytochrome P-450 activity; which may result in increased generation of reactive molecules.

Beside an appropriate dietetic regimen, encouraging although preliminary results have been obtained in patients treated with ursodeoxycholic acid or the insulin-sensitizer drugs metformin. Further researches are required to achieve a better understanding of this condition and to develop definite treatment options.

**Oxidative Balance in Fatty Livers**

Increased generation of reactive oxygen species [ROS] has been observed in several models of fatty livers. Recently, the oxidative stress has been evaluated in rats fed a steatogenic choline-deficient diet. In this model, choline deficiency leads to a fast accumulation of intracellular lipids by blocking the efflux of triglycerides from the hepatocytes, and closely resembles the changes occurring in human fatty liver after excessive dietary intake of carbohydrates. Compared to control animals, rats fed the choline-deficient diet showed significantly lower concentrations of α-tocopherol and vitamin C and higher levels of thiobarbituric acid reactive substances (TBARs), a validated index of lipid peroxidation (Table I). The α-tocopherol/total lipids and α-tocopherol/TBARs ratios were significantly lower than in normal organs. No major difference in the level of glutathione (GSH) was noted. A corroboration to these findings, fatty infiltration of hepatocytes was found to be associated with a lower antioxidant capacity and enhanced risk of lipid oxidation. Indeed, α-tocopherol is the main antioxidant involved in the protection of unsaturated lipids from oxidative modification. Clinical and experimental conditions showing decreased α-tocopherol levels are associated with enhanced peroxidation of lipids and lipoproteins. Therefore, from the results of the above mentioned study it is possible to conclude that high levels of TBARs in fatty livers are associated with impaired lipid protection. It is also known that the redox state of α-tocopherol depends on the availability of reduced ascorbate, which was found also markedly decreased in fatty livers. The maintenance of ascorbate in the reduced form is, in turn, associated with the availability of reduced substrates and GSH-related enzyme activities. Finally, the imbalance between oxidants and antioxidants predisposes fatty livers to a greater injury when exposed to a second insult involving ROS generation.

The ischemia-reperfusion, which invariably occurs during the liver transplantation procedure, represents the clinical application to test this hypothesis. In normal livers, a burst of ROS production occurs during reperfusion following either warm or cold ischemia. ROS damage the cell either directly, by attacking cellular molecules, including lipids, proteins, and nucleic acids or indirectly through the promotion of other damaging mechanisms including the activation of tran-

### Table I. Hepatic concentrations of α-Tocopherol, vitamin C and thiobarbituric acid reactive substances (TBARs) in rats with normal liver and with choline-deficient diet induced fatty liver; total glutathione (GSH) concentration in mitochondria isolated from normal and fatty livers of fed and 18 hours fasted rats.

<table>
<thead>
<tr>
<th></th>
<th>Normal livers</th>
<th>Fatty livers</th>
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<tr>
<td><strong>Liver</strong></td>
<td></td>
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<tr>
<td>α-Tocopherol</td>
<td>120 ± 7</td>
<td>64 ± 8*</td>
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<td>(nmol/g liver)</td>
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<tr>
<td>Vitamin C</td>
<td>117 ± 5</td>
<td>71 ± 4*</td>
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<tr>
<td>(µmol/g liver)</td>
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<tr>
<td>TBARS</td>
<td>2.1 ± 0.2</td>
<td>5.1 ± 0.7*</td>
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<tr>
<td>(nmol/g liver)</td>
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<tr>
<td><strong>Mitochondria</strong></td>
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<tr>
<td>GSH</td>
<td>7.8 ± 0.3</td>
<td>5.2 ± 1.1*</td>
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<tr>
<td>(nmol/mg protein)</td>
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<tr>
<td>Fed</td>
<td>6.7 ± 0.9</td>
<td>4.2 ± 0.7*</td>
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<td>Fasted</td>
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* Significantly different (0.001 < P < 0.05) compared to normal livers.
cription factors for the synthesis of pro-inflammatory cytokines and adhesion molecules.

Experimental observations indicate that fatty infiltration is associated to a greater ROS-mediated liver injury during ischemia-reperfusion. Lipid peroxidation was increased during reperfusion after warm ischemia in rats with fatty livers. Moreover, a short course of tocopherol administration prevented the oxidative stress in fatty livers and improved survival following lethal ischemia.

Recently, by the use of a chemiluminescence apparatus connected to an ultrasonic videocamera with image intensifier, it has been shown that liver steatosis induced by a choline deficient diet increases ROS generation during post-ischemic reoxygenation.

Enhanced oxidative stress represents an important feature in the pathogenesis of NASH. In fact, three main intracellular sites are supposed to be possible sources of ROS in livers with NASH: (1) the cytochrome P 450 CY P 2E1, induced by free fatty acids, generates ROS during the metabolism of endogenous ketones and dietary constituents; (2) peroxisomal \( \beta \)-oxidation, which releases hydrogen peroxide \( \text{H}_2\text{O}_2 \) when mitochondrial \( \beta \)-oxidation is saturated or impaired; \( \text{H}_2\text{O}_2 \) is then converted to highly reactive radicals in the presence of free iron; (3) mitochondria, which continuously generates ROS and are damaged themselves if the production of ROS is increased.

Insulin resistance is believed to stimulate directly the CY P 2E1 activity by the loss of repressive effect of insulin and through upregulation mechanisms common also to peroxisomal \( \beta \)-oxidation. A citration of peroxisome proliferator-activated receptor-\( \alpha \) (PPAR-\( \alpha \)), which regulates both microsomal and peroxisomal lipid oxidation pathways, results in increased production of ROS.

Furthermore, alterations of the intracellular detoxification and radical scavenger systems is present in fatty livers; in fact, both impairment of the transsulfuration pathway and decreased sulphydril content have been reported in fatty livers.

Taken together, these observations suggest that NASH may result from both an increased intracellular generation of ROS and a lower antioxidant defence.

Mitochondrial Abnormalities and Energy Charge in Fatty Livers

Fat accumulation in hepatocytes is accompanied by a number of intracellular disorders as a consequence or cause of excessive lipid infiltration. Prominent abnormalities have been described in mitochondria both in human and experimental steatosis and with the administration of commonly used drugs.

Mitochondrial oxidative metabolism represents the main energy source for the cell. Impairment of specific mitochondrial functions may result in a deficient ATP production and reduced exchange of substances with the cytosol. For instance, oxidative mitochondrial damages observed during reperfusion after warm or cold ischemia may contribute to the deterioration of hepatic energy metabolism observed after transplantation of fatty livers. Thus, the recovery time to achieve normal energy charge after reperfusion is markedly prolonged explaining also the low tolerance of fatty livers to ischemia-reperfusion injury.

In a recent study, the oxidative balance and the capacity for ATP synthesis have been investigated in mitochondria isolated from steatotic livers of rats fed a choline deficient diet. This study showed that mitochondria of fatty livers have a reduced content of GSH, the main organelle antioxidant, a reduced content of protein sulphydrils, which are representative of several enzyme and structural proteins, and higher levels of lipid peroxidation products. By means of polyacrylamide gel electrophoresis of mitochondrial proteins, it was possible to demonstrate a lower intensity of the band representing the ATP-synthase complex. The immunoblot analysis of this band showed a 35% lower detection of the catalytic \( \beta \)-F subunit of the ATP synthase complex. These findings correlated with a significantly decreased content of hepatic ATP.

Patients with NASH exhibit ultrastructural mitochondrial changes, decreased mitochondrial respiration and impaired ATP generation capacity. In patients with steatosis, mitochondrial generation of ROS may oxidatively alters fat deposits; the resulting lipid peroxides may further impair the respiratory chain component and the membrane transport capacity. This vicious circle involves
ROS-mediated antioxidant depletion and impairs the mitochondrial capacity to inactivate ROS. Excessive lipid peroxidation might ultimately trigger the release of cytotoxic cytokines [TNF-α, TGF-β, IL8,...], FAS ligand and stimulate fibrogenesis.

Fasting and Diet Supplementation in Fatty Livers

Obese patients with liver steatosis take advantage of weight loss. Weight reduction also improves liver blood tests, histology and abdominal pain in NA SH patients. However, fatal hepatic failure may follow sudden marked weight reduction in morbidly obese patients with severe NA SH, so that, careful clinical follow-up is recommended in patients undergoing weight-reducing surgery. These clinical observations suggest that fatty livers poorly tolerate excessive food deprivation. In a recent report, rats with fatty livers starved for 18 hours showed an eight fold increase in serum ALT; this finding was absent in rats with normal livers undergoing a similar starvation. Moreover, after normothermic ischemia-reperfusion, the survival rate was adversely affected by fasting much more in rats with fatty liver than in rats with normal liver. Thus, it appears that a well preserved nutritional state can protect the liver against oxidative stress, and indeed conditions depleting the cellular stores of free radical scavengers may increase the vulnerability of steatotic cells to oxidative injury. Prolonged fasting impairs the antioxidant capacity of the liver cells by reducing the availability of amino acids precursor for GSH synthesis. Food deprivation can be even more deleterious in steatotic hepatocytes, which basically show an alteration of the transsulfuration pathway. Indeed, a recent experimental study showed that starvation induces a significantly greater decrease of the hepatic concentrations of α-tocopherol, GSH and vitamin C in steatotic than normal livers. The fall of antioxidant molecules was accompanied by enhanced contents of oxidative products of both lipids and proteins. Interestingly, the deleterious effect of starvation is evident also in the mitochondrial compartment of fatty livers where fasting lowered significantly the GSH concentration (Table I) and altered the ATP-synthase, whose band almost completely disappeared in steatotic livers. This latter finding was accompanied in fatty livers by a 70% decrease of the immunodetected β-F1 subunit and by a 25% reduction of the hepatic content of ATP. Fasting also predisposes mitochondria to a greater oxidative injury during ischemia-reperfusion by further depleting mitochondrial GSH.

These studies show that nutrition is an important determinant of the extent of oxidative injury in the liver either normal and, even more, if fatty. A good nutritional status renders hepatocytes more resistant to ischemic insults and more protected against oxidative stress because of the larger glucogen stores. In this respect, GSH level is likely to play a fundamental protective role, since GSH-depleting substances exacerbate lipid and protein oxidation in liver tissue. Mitochondrial swelling and ultrastructural alterations observed by electron microscopy in food deprived rats during post-ischemic reperfusion finally represent indirect evidence of mitochondrial suffering and dysfunction. Considered together, these findings strengthen the idea that liver parenchymal cell function is deranged only once the endogenous glucose reserves are completely utilised. This means that in the presence of glucogen depletion, hepatocytes are more susceptible to be damaged as it occurs in steatosis. These observations open the way to further investigations suggesting supplementation of substances rather than simple diet restriction as a measure to ameliorate fatty liver response to damaging insults.

Metabolic Differences Among Models and Susceptibility to Necrosis

A series of experimental evidence, as above reported, indicates that livers with steatosis present some disturbances in the hepatic and mitochondrial GSH regulation. Under stress conditions, these organs are further disadvantaged because extremely poor in ascorbate, thus not allowing an antioxidant sparing activity in the presence of low GSH levels. Several metabolic derangement have been identified in fatty livers including subcellular organelles; however, differences seem to exist among the experimental models used to induce liver steatosis. While hepatocyte death is not increased in obese mice under
basal condition, the higher ALT level found in the choline-deficiency model indicates a higher grade of hepatocyte lysis. Difference are also reported at mitochondrial level, where in obese mice mitochondrial GSH content was found increased and the ATP synthase unchanged\textsuperscript{56}, both these parameters were found to be affected in choline-deficient rats\textsuperscript{41}. However, nevertheless these differences in the mitochondrial energy production and antioxidant status, both these models of fatty livers elicited an increased vulnerability to necrosis when challenged by insults which produce minor damages in normal livers. Fatty livers of obese mice probably because their mitochondria appear to be adapted to a chronic increased release of oxygen radicals and promote an increased expression of anti-apoptotic molecules thus resulting hepatocytes protected toward apoptosis\textsuperscript{56}. choline-deficient rats because show an easy loss of cytosolic antioxidants, an inefficient mitochondrial ATP synthesis and a deep loss of mitochondrial GSH under stressing conditions\textsuperscript{10}, all factors associated with a high susceptibility to cell necrosis. These differences clearly suggest the need to investigate the metabolic response to stress conditions in every experimental model of fatty liver.

**Perspectives**

A better understanding of the mechanisms leading to fat accumulation and oxidative balance impairment in steatotic livers is expected to improve the therapeutic approach against the risk to develop NA\textsubscript{SH} and might suggest preventive care to increase the tolerance of these organs toward oxidative stress.

Although diet restriction remains the mainstay of treatment in patients with NA\textsubscript{SH}, encouraging results have been reported with pilot studies evaluating gemfibrozil, ursodeoxycholic acid, N-acetylcysteine, vitamin E and insulin-sensitizer drugs\textsuperscript{12,57,58}. However, these medications still have to be evaluated in carefully controlled long-term studies. Among them, a special attention may deserve the use of drugs able to decrease insulin resistance, which is believed to contribute in these patients to the accumulation of triglycerides in the liver and to the impairment of the fatty acid β-oxidation\textsuperscript{39-61}. Drugs that increase the efflux of triglycerides from the liver or that increase their utilisation, might be another option. Carnitine and coenzyme A, for example, are essential co-factors in the transport of fatty acids into mitochondria and their subsequent oxidation. The need for high dose parenteral administration to reach appreciable hepatic concentrations, however, is against their clinical use, so far.

Based on the evidence reported in the previous paragraphs, any strategy that enables fatty liver to increase the resistance to stress conditions must necessarily improve the basal function of these organs. Thus, a rationale approach aiming to increase the tolerance of steatotic livers to ischemia-reperfusion injury should enhance the hepatic content in antioxidant molecules, enlarge the glycogen reserves and ameliorate their utilisation.

**References**


Diet and fatty liver


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