Point-by-point reply to Reviewer 1

1) Correct redundancies in text and units „ A daily consumption of more than 60-80 g in men and more than 20 g/d in women“

As suggested by the Reviewer, redundancies were omitted and units corrected.

Original, Introduction

Hepatic steatosis is characterized by the deposition of triglycerides in the liver. It is a very frequent condition in adults and obese adolescents in most industrialized countries with ever-increasing prevalence. By definition, in hepatic steatosis more than 5% of hepatocytes contain visible lipid droplets in either a micro- or macrovesicular pattern.

Revision: Introduction

Hepatic steatosis is defined by the accumulation of triglycerides resulting in more than 5% of hepatocytes containing visible lipid droplets in either a micro- or macrovesicular pattern.

Original: Introduction

Steatosis is also frequently found in patients with chronic hepatitis C virus infection. Several drugs have also been reported to potentially cause significant intrahepatic triglyceride accumulation. Acute development of fatty liver disease (AFLP) in late pregnancy is a rare but life-threatening disorder.
Other causes of hepatic steatosis include malnutrition, total parenteral nutrition, hereditary metabolic disease such as glycogen storage disease, Wilsons disease or abetalipoproteinemia, genetic or acquired forms of lipodystrophy or side effects of various surgical procedures such as extensive small bowel resection or biliopancreatic diversion

Revision: Introduction

Beyond metabolic or alimentary causes, fatty liver can also be found in patients with hepatitis C virus infection and rarely in pregnancy. Other rare causes of fatty liver disease are summarized in Table 1.

Original: Paragraph "Alcoholic fatty liver disease"

A daily consumption of more than 60-80 g/d in men and more than 20 g/d in women for more than ten years was shown to cause advanced liver disease in less than 40% of the cases. Susceptibility factors include female sex, obesity, cigarette smoking as well as coexistence of other hepatic disorders such as hepatitis B or C virus infection, non-alcoholic fatty liver disease or hemochromatosis [59].

Revision: Paragraph "Alcoholic fatty liver disease"

Chronic consumption of more than 30 g of pure alcohol was demonstrated to significantly increase the risk of chronic liver disease [60]. Susceptibility factors include female sex, obesity, cigarette smoking as well as coexistence of other hepatic disorders such as hepatitis B or C virus infection, non-alcoholic fatty liver disease or hemochromatosis [61].

2) An additional figure showing the dynamic of lipid droplets formation and accumulation as well as the factors affecting the processes would markedly improve the clarity of the review.

We are very thankful for this suggestion. A figure showing important pathophysiological aspects in development of NAFLD and determinants of lipid droplet morphogenesis was added.

Revision: Figure 1 added

Revision: Non-alcoholic fatty liver disease, last paragraph

Pathophysiological aspects of hepatic triglyceride accumulation in NAFLD are summarized in Figure 1.

Revision, Figure Legend

Pathophysiological aspects of hepatic triglyceride accumulation in NAFLD

Both increased uptake of fatty acids due to elevated whole body lipolysis in states of insulin resistance and enhanced fatty acid synthesis are key features of non-alcoholic fatty liver disease. Increased de novo lipogenesis results from enhanced activation of LXR, SREBP-1c and ChREBP in insulin resistance. SREBP-induced activation of ACC2 leads to accumulation of malonyl CoA, which in turn inhibits CPT-1 activity resulting in reduced β -oxidation. In the liver, triglycerides are stored in LDs which are formed within the lipid bilayer of the ER. Stabilization and growth or LDs is dependent on transmembrane proteins, seipin and triglyceride and phospholipid synthetic enzymes which are located on the LD surface.

LDs are catabolized via ATGL-dependent hydrolysis and β -oxidation of fatty acids, lysosomal lipases and carboxylesterase 3- and CIDEB- mediated repacking of cytosolic LDs in the ER leading to synthesis of VLDL particle.

Intermediates of long chain fatty acids (DAG) inhibit insulin signalling further exacerbating hepatic insulin resistance by exerting proinflammatory effects and reducing activation of the insulin receptor.

3) As suggested a final section on future perspectives of treatment was added. We are very thankful for this suggestion.

Revision, final paragraph

Future Perspectives

Although knowledge on pathophysiology of fatty liver disease has significantly improved in the past years, treatment options especially of NAFLD are still very limited. Several studies suggest beneficial effects of weight loss on course of NAFLD. Pharmacologically, thiazolidinedione (glitazones) and antioxidative Vitamin E are the most promising therapies today. Novel expectant concepts include activation of SIRT-1 (eg reservatrol), which was found to be beneficial in murine models of NAFLD by exerting insulin-sensitizing, antiinflammatory and antioxidative effects [117, 118]. In a very recent work, a liver specific LXR inverse agonist significantly reduced hepatic steatosis by reducing de novo lipogenesis [119]. The effect of Simtuzumab, which is a humanized antifibrotic monoclonal antibody against Lysyl Oxidase Like Molecule 2 (LOXL2) is currently under investigation in patients with adcanced non-alcoholic fatty liver disease [120]. FXR agonists such as obeticholic acid (OCA) are probably the most hopeful and already far advanced therapeutic option under investigation in NAFLD.

Point-by-point reply to Reviewer 2

1) A complete overview of the biochemical pathways involved in the genesis of liver steatosis should also include the aspects related with the glyceroneogenesis (Nye CK, Richard W, Hanson and Satish C. Kalhan. J Biol Chem 2008). Very few authors experts in the field of clinical problems related with liver steatosis know the importance of this biochemical pathway. Authors should write some lines about.

We are very thankful for this constructive and important comment. As suggested data on the role of glyceroneogenesis in fatty liver has now been added.

Revision, „Non-alcoholic fatty liver disease", 2nd paragraph
Further highlighting the role of adipose tissue in triglyceride metabolism, Nye and colleagues [12] found that glycerol 3-phosphate which is essential for triglyceride synthesis primarily originates from glyceroneogenesis and only to a lesser extent from glycolysis.

2) Another comment is on the association between insulin resistance and chronic hepatitis C infection (HCV). It has been suggested a genotype-specific interaction between IR and HCV. I suggest to add some comments about.

We are also very thankful for this very helpful suggestion.

Revision, 1st paragraph: Chronic HCV infection
Interestingly, infection of immortalized hepatoma cell line HepG2 with HCV genotype 1b was associated with suppressor of cytokine signalling 3 (SOCS-3) mediated impairment of insulin signalling when compared to HCV genotype 2 infected cells [92].