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WELCOME MESSAGE

NAFLD has evolved over the last years as the most common liver disease worldwide paralleled by the obesity pandemic. Metabolic and inflammatory aspects have been increasingly recognized to play a crucial role in obesity and related disorders including NAFLD. Besides the key role of metabolic and immune processes as driving forces in these diseases, research from the last years has increasingly suggested that the intestinal microbiota might be critically involved in metabolic inflammation.

The conference will provide all participants a unique opportunity to discuss the recent developments in the field of microbiota, metabolism and NAFLD with excellent speakers and leading authorities.

With warm regards,

SCIENTIFIC ORGANISING COMMITTEE

Herbert Tilg

Michael Trauner
SCIENTIFIC ORGANISING COMMITTEE

Herbert Tilg, Innsbruck, Austria
Michael Trauner, Vienna, Austria

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GENERAL INFORMATION
GENERAL INFORMATION

VENUE
Congress Messe Innsbruck
Congress Center
Rennweg 3
6020 Innsbruck
Austria

INFORMATION ABOUT INNSBRUCK
One of the great things about Innsbruck is that almost every corner you turn affords spectacular views of the Nordkette Alps, the city’s natural skyscrapers. Urban meets outdoors in the relaxed Tyrolean capital, where Zaha Hadid’s space-age funicular speeds you from the centre to alpine pastures clanging with cowbells in a matter of minutes.

Where else can you spend the morning browsing Old Master paintings in Habsburg palaces, the afternoon skiing and the evening in a rollicking brewpub? With a late-medieval Altstadt (old town), vibrant cultural scene and adrenalin-fuelled pursuits of Olympic fame, Innsbruck is one of those rare places that really can say it has something for everyone.

City Web Site: http://www.innsbruck.info/en/home.html

LANGUAGE
The official language of the conference is English.

CLIMATE
The month of February is quite cold, with daily highs increasing from 4°C to 8°C over the course of the month, exceeding 14°C or dropping below 0°C only one day in ten.

NAME BADGES
All participants are kindly requested to wear their name badges throughout the EASL Monothematic Conference in order to be admitted to the lecture halls and other scheduled activities.

REGISTRATION
The onsite registration desk at the conference venue, will be opened:

Thursday, Feb. 26 16.00 - 19.30
Friday, Feb. 27 8.00 - 18.30
Saturday, Feb. 28 8.00 - 12.00

CME ACCREDITATION
The ‘EASL Monothematic Conference: Microbiota, Metabolism and NAFLD’ accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net

The ‘EASL Monothematic Conference: Microbiota, Metabolism and NAFLD’ is designated for a maximum of (or ‘for up to’) 12 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.
Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credit to AMA credit can be found at www.ama-assn.org/go/internationalcme

Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

EVALUATION FORMS
Session Evaluation Forms - The session evaluation forms will be available on the mobile application.

CME Events Evaluation Form - These will be available online. A link will be sent to you by e-mail after the conference. In order to receive a Certificate of Attendance, a CME Events Evaluation Form must be completed online.

Certificate of Attendance - Please note that a completed CME Events Evaluation Form is a pre-requisite in order to receive a Certificate of Attendance. Upon completion of all mandatory online evaluations, the EASL Office will send you an electronic version of your certificate by e-mail.

LIST OF PARTICIPANTS
To be displayed on the notice board located at the EASL Booth.

DRESS CODE
Informal for all occasions.

SMOKING POLICY
This will be a non-smoking event.

BANKING
The official currency in Austria is the Euro (€)

CURRENCY EXCHANGE
Foreign currency can be exchanged at banks, bureau de change and automatic currency exchange machines.

SAFETY & SECURITY
Please do not leave bags or suitcases unattended at any time, whether inside or outside the session halls. Hotels strongly recommend that you use their safety deposit boxes for your valuables.

LIABILITY & INSURANCE
The EASL Office cannot accept liability for personal accidents or loss of or damage to private property of participants. Participants are advised to take out their own personal travel and health insurance for their trip.
LIVER DISEASE IN RESOURCE LIMITED SETTINGS

MAY 29-30, 2015
BUCHAREST, ROMANIA

ABSTRACT SUBMISSION DEADLINE
FEBRUARY 27, 2015

Scientific Organising Committee
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For the scientific programme, online applications, and additional information please visit: www.easl.eu/_events

EASL thanks its Premium Sponsors for their generous contributions and support.
SCIENTIFIC PROGRAMME
DAY I – THURSDAY, FEBRUARY 26, 2015

12:00 – 15:00  REGISTRATION

15:00 – 19:00  I. MICROBIOTA: MISSING LINK IN NAFLD?

**Chairs:** Alexander Moschen, *Austria*
Michael Trauner, *Austria*

15:00 – 15:30  Microbiota and NAFLD: an evolving interaction
Herbert Tilg, *Austria*

15:30 – 16:00  The human gut microbiome: an overview
Petra Louis, *UK*

16:00 – 16:30  Interactions between gut microbiome and metabolic pathways
Fredrik Bäckhed, *Sweden*

16:30 – 17:00  Dysbiosis in obesity, diabetes and NAFLD
Antonio Gasbarrini, *Italy*

17:00 – 17:30  Coffee break

17:30 – 18:00  Dysbiosis in alcoholic fatty liver disease
Vanessa Stadlbauer, *Austria*

18:00 – 18:30  Diet: major confounder of the gut’s microbiota
Gary Wu, *USA*

18:30 – 19:00  Key note lecture 1: Inflammasome, microbiota and NAFLD
Wajahat Mehal, *USA*

19:00  Welcome Reception
### DAY 2 – FRIDAY, FEBRUARY 27, 2015

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<td>Pro- and anti-inflammatory diets in NASH: fructose and beyond</td>
<td>Ina Bergheim, Germany</td>
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<td>Lipotoxicity in NAFLD</td>
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<td>Giulio Marchesini, Italy</td>
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<td>Marc Donath, Switzerland</td>
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14:00 – 18:00  3. METABOLISM, MICROBIOTA AND INFLAMMATION (PART 2)

Chairs:  Mark Hull, UK
         Fabio Marra, Italy

14:00 – 14:30  The impact of gut-flora modification with probiotics and the gut-liver FXR-FGF-CYP7A1-AXIS
Antonio Moschetta, Italy

14:30 – 15:30  ABSTRACT PRESENTATIONS

14:30 – 14:50  Complement factor C3 aggravates non-alcoholic steatohepatitis and affects intestinal microbiota composition in mice
Sander Rensen, Netherlands

14:50 – 15:10  Human gut microbiota affects bile acid metabolism and FXR signalling in gnotobioic mice
Annika Wahlström, Sweden

15:10 – 15:30  The gut microbiota promotes hepatic inflammation and lipid elongation independently on dietary lipid composition
Robert Caesar, Sweden

15:30 – 16:00  Genetics of NAFLD
Christopher Day, UK

16:00 – 16:30  Coffee Break

16:30 – 17:00  NASH: Who develops liver inflammation and fibrosis
Detlef Schuppan, Germany

17:00 – 17:30  Role of the microbiome in hepatocellular carcinoma
Shin Yoshimoto, Japan

17:30 – 18:00  Key note lecture 2: Novel bacterial strains as therapeutics in inflammatory disorders
Willem de Vos, Netherlands
DAY 3 – SATURDAY, FEBRUARY 28, 2015

9:00 – 13:00  4. NAFLD: CLINICAL ASPECTS AND RELATION TO MICROBIOTA

Chairs: Giulio Marchesini, Italy
        Chris Day, UK

9:00 – 9:30  Crosstalks between gut microbes and host cells control metabolism during obesity and diabetes
             Patrice Cani, Belgium

9:30 – 10:00  NAFLD, cardiovascular and cardiac disease
              Christopher Byrne, UK

10:00 – 10:30  NASH and hepatic/extrahepatic cancer
               Mark Hull, UK

10:30 – 11:00  Bariatric surgery, modulation of microbiota and NASH
               Karine Clément, France

11:00 – 11:30  Coffee Break

11:30 – 12:00  Serum metabolomics in NAFLD
               José M. Mato, Spain

12:00 – 12:30  Treatment of NAFLD: modulating the gut microbiota
               Gerardo Nardone, Italy

12:30 – 13:00  Novel therapies for NASH: what is on the horizon?
               Vlad Ratziu, France

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THE LEADING LIVER ASSOCIATION IN EUROPE

APPLY FOR AN EASL FELLOWSHIP PROGRAMME!

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The Dame Sheila Sherlock EASL Fellowship programmes aim to enhance the mobility of investigators within different European institutions, actively promote scientific exchange among research units in Hepatology, and enable physician scientists to take leave from clinical duties to spend 6-12 months in a research laboratory.

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INVITED SPEAKERS’ ABSTRACTS
MICROBIOTA AND NAFLD: AN EVOLVING INTERACTION

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Non-alcoholic fatty liver disease (NAFLD) has emerged as a major health problem in many countries throughout the world paralleling the recent epidemic of “global obesity”. NAFLD is characterized by a broad spectrum of manifestations, ranging from simple steatosis, non-alcoholic steatohepatitis (NASH) to liver cirrhosis and hepatocellular carcinoma. Whereas overnutrition and obesity are crucially involved in the development of a simple fatty liver, it remains unclear why approximately 10% of all affected individuals develop an “inflammatory” phenotype i.e. non-alcoholic steatohepatitis (NASH). A link between the microbiota and development of obesity and NAFLD has recently been suggested. First studies in NAFLD are suggesting that microbiotal factors are driving forces of hepatic steatosis and inflammation involving certain toll-like receptors and pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNFα). Most data so far, however, with respect to microbiota and obesity/NAFLD have focused on animal studies. Evidence has also evolved in the last years suggesting that the intestinal microbiota is involved in human NAFLD. Patients with NASH exhibit small intestinal bacterial overgrowth, an impaired intestinal permeability, and increased circulating endotoxin and TNFa levels. Recently reported clinical studies investigated the gut microbiome in NASH patients. In these studies, differences were abundant at phylum, family, and genus levels between healthy subjects and NASH patients. Proteobacteria, Enterobacteriaceae, and Escherichia were the only phylum, family and genus types exhibiting significant difference between obese and NASH microbiomes. Therefore, evidence is increasing that there exists similar to obesity and type 2 diabetes a “gut microbiotal signature” which might allow in the future to differentiate between patients with simply fatty liver and NASH and might furthermore enable to elucidate underlying pathomechanisms in the development of NASH. Independent of obesity, insulin sensitivity could be triggered in addition by enhanced gastrointestinal permeability, allowing bacterial products to enter circulation. It needs to be established in the future how manipulation of the gut’s microbiota might prove beneficial for patients with NAFLD.
THE HUMAN GUT MICROBIOME: AN OVERVIEW

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The human intestine is populated by a diverse community of microbes that interact with the host in numerous ways, which can have both health-promoting as well as detrimental consequences for host health. The advance in molecular technologies in recent years has led to a detailed characterisation particularly of the bacterial component of the gut microbiota, but progress is also being made in our understanding of its viral and Eukaryotic components. Gut bacteria mostly belong to a few dominant phyla, mainly Firmicutes and Bacteroidetes, and to a lesser degree Actinobacteria and Proteobacteria. Within these phyla there is however a high degree of diversity, and it is estimated that hundreds of different bacterial strains are present within each individual. Some abundant species are found in the majority of healthy people, whereas others are more individual-specific. There have been several attempts at categorising individual microbiota profiles into distinct ‘enterotypes’ and associate those with specific diseases or environmental lifestyles, such as dietary habits. Diet plays an important role in shaping the gut microbiota, as non-digestible carbohydrates, which cannot be broken down in the upper gut by host enzymes, constitute an important carbon and energy source for the microbes in the lower gut.

It is estimated that the microbiome (the collective genomes of the microbiota) contains approximately 100-fold more unique genes than the host genome, thus the microbiota can be regarded as an extra organ of the human body that contributes unique functions to host physiology. Numerous metabolites that are present in host compartments, such as blood or urine, derive from microbial metabolism or an interplay between host and microbial metabolism. The breakdown of non-digestible carbohydrates results in the formation of short-chain fatty acids that are used as energy sources by the host, but also have regulatory effects, ranging from affecting barrier function and inflammatory tone of the gut to modulating host satiety levels. The microbiome also contributes the capacity to synthesize essential vitamins and amino acids, and is involved in the metabolism of numerous compounds entering the host via the diet or other routes, such as plant phenolics or medicines. These metabolic transformations can result in a change of either bioavailability or biological effect of the resulting metabolites.
Furthermore, the microbiota is in close cross-talk with the host’s immune system via several molecular routes, including microorganism-associated molecular patterns (MAMPs) such as lipopolysaccharide or flagellin, that are sensed by host pattern recognition receptors (PRRs).

The gut microbiota is an important contributing factor to both the maintenance of host health and the progression to disease, due to the numerous ways in which it interacts with its host. Its modulation to a more health-promoting state is therefore an attractive target for disease prevention and treatment. However, its complexity and the high level of inter-individual variation, together with possible confounding factors that may affect microbiota composition in parallel to changes in host status, often make it difficult to resolve cause and effect and clearly define what constitutes a healthy microbiota composition. Nevertheless, in the context of non-alcoholic fatty liver disease, certain functions of members of the gut microbiota deserve particular attention. These include its effects on fatty acid metabolism via regulatory pathways, modulation of bile acid and choline metabolism, the endogenous production of ethanol, and changes in inflammatory status.
INTERACTIONS BETWEEN GUT MICROBIOME AND METABOLIC PATHWAYS

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The human gut is inhabited with trillions of bacteria, gut microbiota, that have co-evolved with us and affect our physiology within and outside the gut. Our bodies are exposed to an immense number of bacteria at birth but it is relatively unclear how members of the gut microbiota interact within itself and with the host. Nevertheless the gut microbiota has recently been suggested as a novel contributor to obesity and related comorbidities, such as type 2 diabetes (T2D) and cardiovascular diseases (CVD). We recently found that the gut microbiota is altered in patients with CVD and T2D and that we can classify patients and T2D patients based on the microbiota. Using germ-free mice we have causally linked the gut microbiota to obesity and insulin resistance and have recently found that the gut microbiota modulates adipose inflammation, bile acid signalling, and enteroendocrine cell function. In particular the gut microbiota has profound effects on bile acid metabolism and subsequent signalling through bile acid receptors such as FXR and TGR5. Recent data suggest that the microbiota can induce obesity through an FXR-dependent pathway both by altering the gut microbial composition as well as modulation of FXR signalling in key target tissues such as the liver.
DYSBIOSIS IN OBESITY, DIABETES AND NAFLD

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Obesity and its complications, including diabetes and nonalcoholic fatty liver disease (NAFLD), are increasing in prevalence with the worldwide epidemic of metabolic syndrome. Many genetic and environmental factors have been suggested to contribute to the development of obesity and NAFLD, but the exact mechanisms are not known. Intestinal ecosystem contains trillions of microorganisms including bacteria, Archaea, yeasts and viruses. Several studies support the relationship between the intestinal microbial changes and the pathogenesis of the metabolic syndrome.

Gut microbial composition and functions are strongly influenced by diet. This complex organ seems to affect host metabolic balance modulating energy absorption, gut motility, appetite, glucose and lipid metabolism, as well as hepatic fatty storage. Altered intestinal microbiota (dysbiosis) may stimulate hepatic fat deposition through several mechanisms: regulation of gut permeability, increasing low-grade inflammation, modulation of dietary choline metabolism, regulation of bile acid metabolism and producing endogenous ethanol. In this regard the modulation of gut barrier represent a potential target for novel treatment strategies.

The crosstalk between microbiota dysbiosis and host is mediated through TLR signaling and inflammasome system. Recent evidences suggest the central role of the liver in driving the innate immune response. An impairment of the fine balance between gut microbes and host’s immune system could culminate in the intestinal translocation of bacterial fragments and the development of “metabolic endotoxemia”, leading to systemic inflammation and insulin resistance.

Diet induced weight-loss and bariatric surgery promote significant changes of gut microbial composition, which seem to affect the success, or the inefficacy, of treatment strategies. Regulation of intestinal microbial ecosystem by diet modifications or by using prebiotics or probiotics could reduce intestinal low-grade inflammation and improve gut barrier integrity, thus, ameliorating metabolic balance and promoting weight loss. However, further evidence is needed to better understand their clinical impact and therapeutic use.
DYSBIOSIS IN ALCOHOLIC FATTY LIVER DISEASE

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One third of heavy drinkers develop alcoholic liver disease (ALD), which has a mortality of 20%. Several cofactors, such as endotoxin, gut leakiness or oxidative stress have been accused to attribute to the development of alcoholic steatohepatitis and cirrhosis. Dysbiosis refers to microbial imbalances in the human body, especially in the digestive tract. The concept of dysbiosis as a pathophysiological mechanism is under investigation for several diseases. In this talk the role of dysbiosis and gut leakiness in ALD as well as potential therapeutic targets will be discussed.

The gut barrier consists of several parts: the microbiome, the mucus layer, antimicrobial and secretory proteins, the epithelial cells with tight junctions and the mucosa associated immune system of the digestive tract. The microbiome has structural, metabolic and immunological functions. Changes in the gut microbiota have been described in animal models of ALD and in human studies. Alcohol causes a decrease in beneficial bacterial species (such as lactobacilli) and an increase in potential pathogens (such as enterococci). Overall the bacterial count is increased, leading to bacterial overgrowth of the small bowel, but the diversity, which is important for the resilience of the microbiome, is decreased. Reasons for this are not fully elucidated yet. An increased gut permeability has been described in animal models of ALD and in human studies. Direct (toxic, iNOS mediated) and indirect (capillary damage, inflammation) mechanisms contribute to the observed increase in permeability.

This leads to an increase in bacterial products in the portal and systemic circulation of patients with ALD. These PAMPs reach the liver and cause an inflammatory response, which acts synergistically with alcohol to damage the liver.

Recently the role of bile acids in the development of ALD has been studied in detail. Alcohol and chronic liver disease changes the bile acid profile and bile acid composition is related to microbiota changes. Since bile acids play a role in endotoxin absorption, modulate intestinal permeability, are ligands of FXR and TGR5, and inhibit bacterial proliferation, these changes may be of pathophysiological relevance.

Potential future therapeutic strategies include probiotic modulation of the microbiome and/or gut permeability, bile acid modulation or nutritional strategies (oats supplementation, modulation of dietary fats).

In summary dysbiosis is of pathophysiological relevance in ALD. Together with increased permeability and the increase of bacterial products in the circulation, dysbiosis leads to more severe liver disease. Therefore, modulation of the microbiome and/or gut permeability is a promising future concept in the treatment of ALD.
DIET: MAJOR CONFOUNDER OF THE GUT’S MICROBIOTA

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The human gut contains a vast number of microorganisms known collectively as the “gut microbiota”. Despite its importance in maintaining the health of the host, growing evidence suggests the gut microbiota may also be an important factor in the pathogenesis of various diseases, a number of which have shown a rapid increase in incidence over the past few decades. In some of these diseases the microbiota is “dysbiotic” with an altered community structure and decrease in diversity. If the dysbiotic microbiota plays a role in disease pathogenesis, interventions that modify its composition might be a strategy to treat certain disease processes. The composition of the microbiota can be influenced by many factors including age, genetics, host environment, and diet. Diet has an impact upon both the composition and function of the microbiota in part through small molecule production that may influence development of both immune-mediated with metabolic diseases. The steady state level of these plasma metabolites can be influenced, not only by their rate of production by the gut microbiota, but also by their absorption and excretion. Elevation of certain metabolites due to decreased renal clearance may play a role in the development of co-morbidities observed in patients with chronic kidney disease such as coronary vascular disease. Finally, by comparing dietary intake, the gut microbiota, and the plasma metabolome in omnivores vs. vegans, we provide evidence that the production of certain bacterial metabolites is constrained by the composition of the gut microbiota. These findings were confirmed in a controlled human diet experiment. In total, these results demonstrate the potential promise of dietary manipulation of the gut microbiota and its metabolome as a modality to both maintain health and treat disease. In order to accomplish this goal, there is a need for human intervention studies to demonstrate cause-and-effect relationships.

Disclosure of Interest: G. Wu Grant: Nestlé; Consultant/Advisor: Janssen, Chr. Hansen, Rebiotix, Other: Co-Founder of Microbiota Therapeutics, LLC
KEY NOTE LECTURE I: INFLAMMASOME, MICROBIOTA AND NAFLD

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NAFLD and NASH are defined by a spectrum of changes which range from hepatocyte steatosis and ballooning to inflammation, injury and fibrosis. These changes cover a diverse range of biological processes, and are influenced by processes outside the liver including changes in the microbiome. A key step in the progression to NASH is the development of liver inflammation, which induces hepatocyte steatosis, apoptosis and insulin resistance. The development of inflammation requires assembly of a set of cytosolic molecules loosely termed the inflammasome. When these are assembled in sequence they result in the activation a cysteine protease (caspase-1) which finally cleaves, activates and releases IL-1b and IL-18. The requirement for inflammasome activation in NASH fits very well into our understanding of inflammation in other forms of sterile inflammation in the liver and other organs, including the development of alcoholic steatohepatitis and ischemia reperfusion injury. The inflammasome machinery is present in most cells, but for liver inflammation activation in Kupffer cell is likely the most important

Identification of a requirement of the inflammasome machinery for NASH provides a very important foothold in this complex area, because much is known about the two types of signals required for inflammasome activation. One set of signals (signal 1) are ligands for pattern recognition receptors (PRR) and include molecules of bacterial origin such as LPS and unmethylated DNA. In a number of models of liver inflammation there is an increase in ligands for PRR in the portal and systemic circulation, and this is due to changes in the number and species of the bacteria, and an increase in intestinal permeability. The second type of signals are very diverse and include particulates such as cholesterol crystals, and a wide range of cell intrinsic molecules that are released in NASH upon cell death (damage associated molecular patters-DAMPs). Most of these DAMPs are likely coming from hepatocytes that have been damaged by metabolic syndrome associated changes such as high levels of free fatty acids, and the production of reactive oxygen species. There is very likely a synergy between signals originating from the microbiome, and the metabolic syndrome which results in the full NASH phenotype.
Inflammasome activation however has consequences in addition to initiating liver inflammation. Caspase-1 activation has been shown to induce a pro-inflammatory form of cell death (pyroptosis), inactivate enzymes involved in glycolysis, activate sterol regulatory binding proteins (SRBP) and stimulate unconventional release of a number of cytosolic molecules lacking a signal peptide. The role, if any, of these process in the development of NASH is unclear. Very relevant to NASH however is the fact that there is a requirement for the NLRP6/ASC/Caspase-1 pathway for normal mucus production by goblet cells in the colon. The absence of any of these molecules results in the loss of the inner mucus layer, a dysbiotic microbiome that is intimately associated with the colonic epithelium, resulting in increased levels of PRR ligands in the portal blood. The microbiome is likely to have many effects on the steatotic liver in addition to providing ligands for PRR. Microbiome metabolites that enter the liver through the portal vein have the potential to regulate liver inflammation. For example, we have recently demonstrated that β-hydroxybutyrate can downregulate inflammasome activation via the GRP109A receptor on Kupffer cells.

In summary experimental data supports a model in which molecules released into the portal circulation by an abnormal microbiome synergize with signals from injured hepatocytes to activate the inflammasome resulting in steatosis, inflammation and fibrosis. It is however clear that the NASH phenotype develops in rodents subsequent to a wide range of experimental manipulations, suggesting caution in concluding which of these mechanism are responsible for NASH in humans.
PRO- AND ANTI-INFLAMMATORY DIETS IN NASH: FRUCTOSE AND BEYOND

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Despite intense research efforts mechanisms associated with the onset and even more so the progression of non-alcoholic fatty liver disease (NAFLD) to later stages of the disease like non-alcoholic steatohepatitis (NASH) or even fibrosis are still not fully understood. With still increasing prevalence, in Europe approximately 3-10% of the general adults population are affected by the disease by now. Besides life style interventions, which are frequently afflicted with low therapy adherence and high relapse rates, therapeutic and preventive options are still limited and universally accepted treatment strategies have not yet been established. Results of several human and animals based studies suggest that not only an increased fat and cholesterol intake but also the intake of certain carbohydrates like fructose may be critically associated with the development of NAFLD. Indeed, it even has been shown that patients with NAFLD not only frequently have a higher overall energy intake but also consume more fructose-rich foods. It further has been suggest that an increased fructose intake may increase the odds to develop the later stages of the disease (e.g. fibrosis). Furthermore, several studies suggest that besides lifestyle and genetic factors an increased permeation of bacterial endotoxin may be involved in the pathogenesis of NAFLD. For instance, it has been shown that patients with different stages of NAFLD suffer from endotoxemia associated with an increased intestinal permeability and a loss of tight junction proteins as well as increased expression of the Toll-like receptors (TLR) and depending signalling cascades in liver tissue. Results of animal studies feeding a diet rich in fructose lend further support to the hypothesis that an increased translocation of bacterial endotoxins from the gut and the subsequent activation of TLR-4-dependent signalling cascades in the liver might be critical factors in the development of NAFLD. In these studies it has been shown, that a chronic intake of fructose was associated with a loss of tight junction proteins in the upper parts of the small intestine, elevated levels of endotoxin in the portal vein and an induction of TLR-4-dependent signalling cascades in the liver. Similar results were also found for animal models using high fat diets, suggesting that both, a diet rich in fructose or fat and maybe even more a combination of both may be critical in the development of NAFLD. It was further suggested dietary interventions focusing a reduction of sugar intake but enhancing the intake of probiotics and certain other nutrients (e.g. secondary plant derived compounds) may be beneficial not only in regards to liver damage but also endotoxin levels. Taken together, altering intestinal microbiota and permeability through dietary modifications or the intake of probiotics maybe beneficial in the treatment of NAFLD.
LIPOTOXICITY IN NAFLD

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Non-alcoholic fatty liver disease (NAFLD) comprises a diverse disease spectrum ranging from ‘simple’ (and relatively stable) steatosis to the potentially progressive steatohepatitis (NASH) with risk for development of advanced fibrosis, cirrhosis and hepatocellular cancer (HCC). Understanding the cellular and molecular mechanisms underlying disease progression may be essential for development of potential diagnostic/prognostic biomarkers and effective pharmacological strategies. Lipotoxicity may be a key mechanism for disease progression and can result from accumulation of lipid intermediates in non-adipose tissues such as liver, leading to cellular dysfunction and cell death triggering inflammation, fibrogenesis and carcinogenesis. Free fatty acids (FAs) resulting from breakdown of triglycerides (TGs) are potential lipotoxic mediators and triggers of inflammation. As such, saturated FAs induce hepatocyte death, while polyunsaturated FAs (PUFAs) exert anti-inflammatory and anti-steatotic effects. In addition to FAs, diacylglycerol (also deriving from TG breakdown) and cholesterol may also be an important lipotoxic triggers for NAFLD progression.

Metabolic lipases such as adiponutrin (PNPLA3), its closest relative adipose triglyceride lipase (ATGL/PNPLA2) and hormone sensitive lipase (HSL) are key enzymes involved in TG breakdown into FAs and glycerol. As such these enzymes may critically determine lipotoxicity through modulation of lipid fluxes and lipid partitioning between adipose tissue and the liver, as well as within liver/hepatocytes. Genetic variants of PNPLA3 have been convincingly linked to pathogenesis of NAFLD and its progression to fibrosis and HCC. Interestingly, PNPLA3 appears to have both lipase and lysophosphatidic acid acyl transferase (LPAAT) function and thereby may be involved in both the synthesis and breakdown of TGs. Insulin resistance results in enhanced lipolysis in WAT via ATGL which creates an increased free FA flux to the liver and thereby promotes lipotoxicity. Moreover, metabolic lipases also provide FAs as ligands for nuclear receptors such as PPARα which control both lipid metabolism and inflammation. Apart from TG hydrolysis in adipose tissue (accounting for 60%) about 25% of free FAs come from increased lipogenesis which is driven by insulin or dietary factors such as fructose. Only a minor proportion of free FAs is derived directly from food.
Dysbiosis in NAFLD/NASH may critically determine hepatic lipotoxicity by impacting on energy extraction and processing/storage of nutrients such as FAs and glucose. Short-chain FAs (SCFAs) derived from fermentation of dietary fibers/polysaccharides by anaerobic intestinal microbiota exert multiple beneficial effects on energy metabolism, intestinal permeability and innate immunity. Moreover, microbiota alter phospholipid metabolism and clearance of serum lipids by lipoprotein lipase, e.g. impacting on FA delivery to adipose tissue. Finally, microbiota modify bile acid (BA) signaling as important modulator of hepatic lipid and glucose metabolism by BA deconjugation and dehydroxylation with formation of more hydrophobic secondary BAs. Further studies will have to explore the potential relationship between key metabolic lipases (e.g. PNPLA3 variants) and intestinal microbiota in determining lipotoxicity in progression of NASH.
INSULIN RESISTANCE IN NAFLD: CLINICAL EVIDENCE

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The association of NAFLD with insulin has long been characterized. Insulin resistance is characterized by increased circulating insulin concentration (reflecting increased need of insulin for peripheral glucose disposal), high free fatty acid levels (reflecting impaired insulin-mediated suppression of lipolysis), as well as impaired lipid oxidation, and high branched-chain amino acids (reflecting both an impaired anabolic effect of insulin and a defect of the anti-catabolic action of insulin). All these metabolic abnormalities are common to NAFLD, as well as to obesity and type 2 diabetes [1-3], and are the basis for the inclusion of NAFLD inside the features of the Metabolic (insulin-resistance) Syndrome [4]. From a clinical point of view, the best evidence for insulin resistance as the background metabolic abnormality responsible for liver fat accumulation stems from the tight association between liver fat accumulation/reduction and body weight gain/loss [5], which also drives the prevalence of type 2 diabetes, cardiovascular risk profile and inflammatory markers (high-sensitive C-reactive protein levels and pro-inflammatory cytokines (IL-1, IL-6 or TNF-α)). Nevertheless, in a limited number of cases this association does not hold true. Lean NAFLD patients rarely present with insulin resistance and glucose intolerance, but exhibit higher levels of alanine/aspartate aminotransferases (ALT/AST) [6]. However, when present, the sites and mechanism(s) of insulin resistance are not different between non-obese, non-diabetic subjects with NAFLD and those with obesity and diabetes [7]. In addition, insulin resistance-associated lipid accumulation per se does not necessarily cause NAFLD [1], or, at least, it is not necessarily involved in disease progression [8]. This dissociation between NAFLD and insulin resistance could be due a variety of mutations, which are the subject of intense research. Notably, it has been recently reported that, despite a healthier metabolic profile, less insulin resistance, and less advanced fibrosis at histology, patient with “lean” NAFLD have a higher overall mortality than patients with NAFLD who are overweight or obese [9]. A reconsideration of the role of insulin resistance in these cases is thus needed.
References


ROLE OF INFLAMMATION IN TYPE 2 DIABETES

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Onset of Type 2 diabetes occurs when the pancreatic beta-cell fails to adapt to the increased insulin demand caused by insulin resistance. Morphological and therapeutic intervention studies have uncovered an inflammatory process in islets of patients with Type 2 diabetes characterized by the presence of cytokines, immune cells, beta-cell apoptosis, amyloid deposits, and fibrosis. This insulitis is due to a pathological activation of the innate immune system by metabolic stress and governed by IL-1 signalling. We propose that this insulitis contributes to the decrease in beta-cell mass and the impaired insulin secretion observed in patients with Type 2 diabetes. Initially, the inflammatory response is probably deployed to promote beta-cell repair and regeneration. Yet, as it becomes chronic activation of auto-inflammatory processes may then become deleterious. Furthermore, IL-6 emerges as an additional regulator of islet secretory function. Indeed, IL-6 mediates a cross talk between fat, muscles and pancreatic islets to adapt to changes in insulin demand via secretion of the incretin glucagon-like peptide-1. Thereby, IL-6 reprograms the alpha cells to produce glucagon-like peptide-1. This implicates IL-1 beta and IL-6 in the regulation of beta cell insulin secretion in both health and disease. It follows that modulation of inflammatory mediators, may present as a possible causal therapy with disease-modifying potential. Several conditions that are driven by inflammatory processes are also associated with diabetes, including rheumatoid arthritis, gout, psoriasis and Crohn’s disease, and various anti-inflammatory drugs have been approved or are in late stages of development for the treatment of these conditions. We will discuss the rationale for the use of some of these anti-inflammatory treatments in patients with diabetes and what we could expect from their use. Future immunomodulatory treatments may not target a specific disease, but could instead act on a dysfunctional pathway that causes several conditions associated with the metabolic syndrome.
ADIPOKINES AND METABOLIC LIVER DISEASE

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Accumulating data demonstrate that obesity and insulin resistance are associated with a more severe and faster progression of the fibrogenic process in different chronic liver diseases, and attention has focused on possible links between the adipose tissue and liver repair.

Adipokines are polypeptides secreted in the adipose tissue in a regulated manner. Leptin, adiponectin and resistin are the best-studied molecules in this class, but cytokines such as CCL2, tumor necrosis factor or interleukin-6 are also secreted at high levels by the adipose tissue. Several other molecules have been recently identified, including chemerin, vaspin, retinol binding protein-4 and apelin, which are being actively investigated. Most of the available data focus on leptin and adiponectin.

Leptin level increase along with adiposity. This adipokine has been extensively characterized for its proinflammatory and profibrogenic role, and several cell types contribute to this action. Leptin directly targets hepatic stellate cells (HSC) via activation of ObRb, triggering a downstream cascade that includes ERK1/2, PI3K/Akt, mTOR and HIF-1. Of note, the actions of leptin on HSC also include activation of NADPH oxidase and ROS production, and phagocytosis of apoptotic bodies. More recently, leptin has been connected to adaptive immunity, the Hedgehog pathway, cannabinoid metabolism and the pathogenesis of portal hypertension.

Adiponectin binds at least two specific receptors, AdipoR1 and AdipoR2. The main downstream effector of AdipoR1 is AMP-activated protein kinase (AMPK), while AdipoR2 signals via peroxisome proliferator-activated receptor-gamma (PPAR-gamma). Adiponectin reduces inflammation in several models of liver injury, and conversely, inflammation blocks adiponectin secretion. Moreover, adiponectin increases insulin sensitivity acting on different tissues. Adiponectin knockout mice develop more extensive fibrosis than wild type animals after chronic CCl4 intoxication, demonstrating that adiponectin has antifibrogenic effects independently of metabolic actions. Reduced fibrogenesis is mediated at least in part by modulation of the activated phenotype of HSC, which express both adiponectin receptors. Activation of AMPK has been identified as a pivotal mechanism mediating the antifibrogenic effects of adiponectin. Another mechanism recently identified is the upregulation of the TGF-b coreceptor, Bambi, which reduces profibrogenic signals. Recently, oral agonists of the adiponectin receptors have been tested in preclinical studies. Adiponectin has been also involved in the biology of hepatocellular carcinoma cells.
HEPATIC MICROBIOTA AND STEATOSIS

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Metabolic diseases are characterized by numerous co-morbidities such as a fatty liver disease (NAFLD). The origin of the excessive lipid storage process is unknown but could be related to a change in gut microbiota. We explored this hypothesis and showed that upon the control of gut microbiota by antibiotics, pre and probiotics impacted the development of fatty liver diseases. Lipopolysaccharides are bacterial molecules accumulating in the blood in response to a fat-enriched diet-induced NAFLD. Their continuous infusion at low rate triggered NAFLD in normal chow fed mice. Importantly, in addition to the accumulation of LPS into the blood we characterized that the fat-enriched diet procedure increased the content in bacterial DNA into the blood but also into the liver. The treatment of mice with a prebiotic impacted the gut microbiota ecology as well as the liver bacterial DNA content and the fat accumulation. Sequencing of liver microbiota revealed specific bacterial DNA and taxa characterizing the liver phenotype. Hence, this first set of experiment paves the way for the discovery of tight molecular relationships between liver microbiota and NAFLD.
THE IMPACT OF GUT-FLORA MODIFICATION WITH PROBIOTICS AND THE GUT-LIVER FXR-FGF-CYP7A1-AXIS

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Gut microbiota influences host health status by providing trophic, protective, and metabolic functions, including bile acid (BA) biotransformation. Microbial imprinting on BA signature modifies pool size and hydrophobicity, thus contributing to BA enterohepatic circulation. Microbiota-targeted therapies are now emerging as effective strategies for preventing and/or treating gut-related diseases. Here, we show that gut microbiota modulation induced by VSL#3 probiotics enhances BA deconjugation and fecal excretion in mice. These events are associated with changes in ileal BA absorption, repression of the enterohepatic farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis, and increased hepatic BA neosynthesis. Treatment with a FXR agonist normalized fecal BA levels in probiotic-administered mice, whereas probiotic-induced alterations in BA metabolism are abolished upon FXR and FGF15 deficiency. Our data provide clear in vivo evidence that VSL#3 probiotics promote ileal BA deconjugation with subsequent fecal BA excretion and induce hepatic BA neosynthesis via downregulation of the gut-liver FXR-FGF15 axis.
COMPLEMENT FACTOR C3 AGGRAVATES NON-ALCOHOLIC STEATOHEPATITIS AND AFFECTS INTESTINAL MICROBIOTA COMPOSITION IN MICE

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Introduction: Bacterial molecules can be recognized by complement factors, thereby promoting inflammation. Previously, we reported the accumulation of activated complement proteins around steatotic hepatocytes in human nonalcoholic steatohepatitis (NASH); this was strongly correlated to the extent of hepatic inflammation.

Aims: We now aimed to assess the role of the central complement component C3 in the development of NASH in relation to its effects on gut microbiota composition.

Material and Methods: C3+/− (WT) and C3-deficient (C3−/−) C57BL/6J mice were fed a high fat diet (HFD) for 3 months or a methionine-choline-deficient (MCD) diet for 6 weeks to induce mild or severe NASH. The development of hepatic steatosis (grade 0-3), inflammation (grade 0-3), and fibrosis (grade 0-2) was compared to that observed in chow fed WT and C3−/− mice. Hepatic inflammation was further characterized by flowcytometric analysis of infiltrated leukocytes and by assessing mRNA expression of specific cytokines and chemokines by qPCR. Fecal microbiota composition was investigated by qPCR and pyrosequencing of 16S rDNA.

Results: Hepatic steatosis was substantially reduced in C3−/− mice on HFD (0.59±0.09 vs. 1.00±0.23 for WT, p<0.05). Surprisingly, lobular inflammation grade was similar in WT and C3−/− mice on either HFD or MCD. However, hepatic NKT cell abundance was markedly reduced specifically in C3−/− mice on MCD (13.45±1.89 vs. 7.08±0.51 for WT, p<0.01). Moreover, liver TNF-α and Mcp-1 expression were lower in C3−/− mice on MCD (4.78±0.61 vs. 6.75±0.76 for WT, p≤0.05; 9.39±1.24 vs. 4.90±1.03 for WT, p<0.05). Whereas WT mice displayed moderate hepatic fibrosis after MCD feeding, fibrosis was undetectable in C3−/− mice on MCD (0.54±0.14 vs. 0.0, p<0.01).
Gut microbiota clustered according to C3 genotype (ANOSIM; $r^2=0.94$, $p=0.001$); this clustering was more pronounced in the HFD condition. C3$^{-/-}$ animals showed a particularly strong reduction of *Akkermansia muciniphila*, which has been shown to increase insulin sensitivity. Furthermore, Shannon’s diversity index was lower in C3$^{-/-}$ mice than in WT mice on HFD.

**Conclusions:** Complement factor C3 appears to promote hepatic steatosis, inflammation, and fibrosis, thus aggravating the development and progression of NASH in mice. This might be related to effects of C3 on gut microbiota given the recent evidence that intestinal bacteria drive metabolic disease.
HUMAN GUT MICROBIOTA AFFECTS BILE ACID METABOLISM AND FXR SIGNALLING IN GNOTOBIOTIC MICE

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Introduction: The influence of gut microbiota on the development and progression of metabolic diseases may be mediated through bile acids (BAs) and their receptor farnesoid X receptor (FXR). Mice colonized with a human gut microbiota are powerful tools to study the effect of human bacteria on metabolic functions but it has been suggested that human bacteria lack the capacity to metabolize the primary murine BA tauro-beta muricholic acid (TβMCA), which is a naturally occurring FXR antagonist in mice.

Aims: In this study we aimed to investigate the impact of a human gut microbiota on BA metabolism and FXR signalling in gnotobiotic mice and in addition we compared fresh and frozen human donor samples.

Material and Methods: Germ-free (GF) mice were colonized with bacteria from human faeces (CONV-H) or mouse caecum (CONV-M). BAs were analysed with UPLC-MS/MS and short chain fatty acids (SCFAs) with GC-MS. Expression of FXR target genes in liver and ileum was analysed with qPCR. Bacterial DNA was extracted from caecum of the colonized mice and the V4 region of the 16s rRNA was amplified and sequenced using the MiSeq Ilumina platform.

Results: Colonization with human microbiota decreased the BA pool but did not change BA composition in gallbladder compared with GF mice. The ratio between the FXR antagonist TβMCA and FXR agonists, tauro-cholic acid (TCA) and cholic acid (CA), measured in gallbladder and portal vein serum was not decreased to the same extent in CONV-H mice as in CONV-M mice, which was reflected by the minor increase in Fgf15 expression. Gut microbiota of CONV-H and CONV-M mice was different in terms of abundances of specific microbial groups. Interestingly, when frozen human faeces were used as donor, the TβMCA/(TCA+CA) ratio was decreased and the Fgf15 expression was significantly increased compared with fresh human faeces.

Conclusions: Human gut microbiota sufficiently reduces the BA pool but has limited effects on BA composition and FXR signalling in gnotobiotic mice. Colonization with frozen human faeces results in an altered microbiota composition compared with fresh faeces in the recipient mice and increases the expression of FXR target genes in ileum.
THE GUT MICROBIOTA PROMOTES HEPATIC INFLAMMATION AND LIPID ELONGATION INDEPENDENTLY ON DIETARY LIPID COMPOSITION

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Introduction: Dietary lipids and gut microbiota affect hepatic physiology in many ways.

Aims: Here we investigate how dietary lipids influence gut microbiota ecology and how the gut microbiota affect liver inflammation and lipid metabolism in the presence of lard (saturated) or fish (unsaturated) oil diet.

Material and Methods: Conventionally raised (CONV-R) and germ-free (GF) C57Bl/6 mice were fed irradiated isocaloric diets (45% kcal fat) with either lard or fish oil. Microbiota composition was determined by 16S rRNA pyrosequencing. Hepatic gene expression was measured by microarray analysis using Affymetrix GeneChip® ST Arrays. Lipidomics analysis of liver and serum samples was performed by UHPLC-MS.

Results: We showed that dietary lipid composition influences gut microbiota ecology with increased levels of bacterial taxa associated with an inflammatory phenotype present in mice fed lard. However, functional analysis revealed that the gut microbiota increases expression of hepatic genes associated with inflammation, both in mice fed lard and in mice fed fish oil. Lipidomics analysis showed that the gut microbiota had only minor effect on lipid abundance on class level. Coherent with this observation, expression of key enzymes involved in lipid biosynthesis was unaffected by the gut microbiota. In contrast, many lipids were regulated on species level, with increased abundance of lipids with very long carbon chains in CONV-R mice compared with GF mice. Notably, CONV-R mice had increased expression of the fatty acid elongase Elovl3 in the liver, potentially explaining the shift in lipid species abundance.

Conclusions: We show that even though dietary lipids have profound influence on gut microbiota composition, the presence of a gut microbiota induces inflammation and increases abundance of lipids with long carbon chains regardless of dietary lipid composition.
GENETICS OF NAFLD

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Non-alcoholic fatty liver disease (NAFLD) is a spectrum of disease ranging from simple steatosis, through steatohepatitis to fibrosis and ultimately cirrhosis and hepatocellular carcinoma. This condition is characterised by significant inter-patient variability in terms of severity and rate of progression: whilst a substantial proportion of the population are at risk of progressive disease, only a minority experience associated morbidity. As such NAFLD is best considered a complex disease trait resulting from environmental exposures acting on a susceptible polygenic background comprising multiple independent modifiers. Environmental factors considered to be important include diet and physical activity and the gut microbiome may also play a role. Until recently studies focussed on identifying the genetic factors that contribute to NAFLD pathogenesis have been restricted to largely unreplicated candidate gene studies. However recent genome-wide association studies, have identified several replicated genetic modifiers of disease progression including PNPLA3 and TM6SF2. The mechanisms of these associations and their implications for treatment and clinical management strategies are currently subjects of intensive study.
NASH: WHO DEVELOPS LIVER INFLAMMATION AND FIBROSIS

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The natural history of the progression of NAFL to NASH and to cirrhosis is less predictable than that of most other chronic liver diseases, such as viral hepatitis B and C (1). In view of the high prevalence of NAFL in most countries, this presents a challenge and the need to develop better (noninvasive) diagnostic and prognostic biomarkers of liver inflammation, fibrosis and especially fibrogenesis has taken center stage in recent consensus conferences (2-3). These meetings have also confirmed that, despite its invasiveness and sampling variability, at present liver biopsy remains the gold standard for the prognostic assessment and diagnostic grading and staging of NASH. Notably, it is not the severity of inflammation but the type of inflammation that drives fibrosis progression, whereas the extent of hepatocyte lipoapoptosis (histological ballooning) is tightly linked to fibrogenesis. However, even patients with NASH and fibrosis appear to display phases of disease progression and regression that are not captured by current methodology. This difficult to predict course of the disease is due to the more recent notion that inflammation and fibrosis in NASH are fueled by several “second hits” on the basis of a more or less steatotic liver, with steatosis being a necessary but not sufficient precondition. While “second hits” promote and exacerbate almost all diseases, this paradigm holds especially true for NASH and fibrosis, since here numerous overlapping pathologies can contribute to overall disease severity. This opens the unique opportunity to prevent and beneficially modulate the course of NASH and fibrosis by relatively simple measures, with specific pharmacological agents serving as adjunctive treatment. Thus apart from a genetic predisposition, such as the PNPLA3 polymorphism, most other determinants/second hits can be modified. This is highly relevant, since even modest modulation of some of these factors, e.g. modest life style changes, can dramatically influence NAFL progression or regression. Different from other liver diseases, efficient treatment of NASH needs to address 1) the metabolic derangement, including insulin resistance/type 2 diabetes, cardiovascular pathology as determinants of the metabolic syndrome, 2) the liver inflammatory component and 3) fibrosis, which show only a partial overlap.
Therefore, therapies that address hepatic steatosis may increase hepatitis and fibrosis as exemplified by inhibition of diacyl glycerol acyl transferase (4), and effective suppression of steatohepatitis may worsen peripheral adiposity as with the glitazones, or increase LDL as for obeticholic acid (5). Apart from specific antifibrotic therapies that are already entering early clinical trials (6-8), other interventions promise to prove antifibrotic as well, although specific evidence is scarce. There is no controversy over the beneficial role of (controlled) weight loss and physical exercise, both improving insulin sensitivity. There is also much plausibility in the relevance of the gut-liver axis, which puts nutrition and the microbiome center stage in NASH (and fibrosis) development. Here activation of toll like receptor 4 (TLR4) via bacterial lipopolysaccharide (LPS) that enters the liver via intestinal barrier defects or via intestinal myeloid cells activated by nutritional amylase trypsin inhibitors (ATIs) ingested with wheat promote both NASH and liver fibrosis (9,10). Nutrients and the host’s intestinal and liver immune system also interact with and are conditioned by the intestinal microbiota, which can promote or ameliorate insulin resistance, NASH and likely liver fibrosis (11). A better understanding of the quantitative contribution of these variant environmental and life-style factors, combined with better noninvasive biomarkers, on fibrosis progression and reversal will permit a better prediction of disease severity and progression, and likely prove to be the most effective preventive and therapeutic measure to treat fibrotic NASH and associated comorbidities.
References


KEYNOTE LECTURE 2: NOVEL BACTERIAL STRAINS AS THERAPEUTICS IN INFLAMMATORY DISORDERS

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Since early life, microbes dominate our body in numbers. In the intestinal tract they constitute the largest microbial ecosystem that is close to our heart: our microbes inside. Previous molecular studies have shown that the intestinal tract microbiota is highly personalized. We now have confirmed and extended this in comparative studies with close to 10,000 subjects that show all a different microbial composition. However, we could detect a conserved core network of functionally related microbes, high-level enterotype-like structures, and specific abundance distributions in healthy Western adults. The latter provides support for the presence of alternative stable states with bistable groups reflecting tipping elements [1]. We have proposed that these states are instrumental for further defining microbiota aberrations, and these tipping points can be used to delineate early warning signals associated with health changes, including inflammatory and metabolic diseases.

Many of the core microbes have been isolated and described, amounting to over 1000 species, although some more await culturing, the exact number depending on their definition [2]. This is of great importance as their availability determines the development of defined microbial therapies. The use of microbial therapies is gaining increasing attention with the success of fecal transplantation in inflammatory and metabolic diseases [3,4]. This contribution will address the recent developments in using defined bacterial strains as therapeutics in inflammatory and metabolic diseases. Specific attention will be given to (i) the mucus-degrading Akkermansia muciniphila that has been found to improve the intestinal barrier function in mice on a high fat diet [5]; (ii) the butyrate-producing Eubacterium hallii that was been identified via reversed engineering following fecal transplantation in metabolic syndrome (4); and (iii) bacteria belonging to a new genus Intestinimonas that are capable of producing butyrate in a novel pathway and have great potential to be developed as new therapeutics. Finally, the use of these and other intestinal bacteria in synthetic communities will be discussed [6].
References:


ROLE OF THE MICROBIOME IN HEPATOCELLULAR CARCINOMA

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Obesity has become more prevalent in most developed countries over the past few decades, and is increasingly recognized as a major risk for several common types of cancer. Although weight loss by exercise and/or dietary control ameliorates obesity-induced metabolic syndromes, the worldwide obesity epidemic has shown no signs of abating. Therefore, more effective methods are needed to prevent obesity-associated cancer development. Toward this purpose, a better understanding of the mechanisms underlying obesity-associated cancer is urgently required. Although it has been proposed that obesity-associated inflammation contributes to cancer development, the exact molecular mechanisms that integrate these events have remained largely unclear. Recently, we showed that obesity-induced cellular senescence and senescence-associated inflammation promote to cancer development. Cellular senescence is a process occurring in normal cells in response to telomere erosion or oncogene activation, acting through checkpoint activation and stable cell cycle arrest as a barrier to tumorigenesis. Recent studies, however, reveal that senescent cells also develop a secretory profile composed mainly of inflammatory cytokines, chemokines and proteases, a typical signature termed the senescence-associated secretory phenotype (SASP). Some of the SASP factors display cell autonomous activities to reinforce senescence cell cycle arrest and/or promote clearance of senescent cells. But, other SASP factors exhibit cell non-autonomous functions associated with inflammation and/or tumorigenesis promotion, indicating that SASP contributes positively and negatively to cancer development, depending on the biological context. We found that SASP has crucial roles in promoting obesity-associated hepatocellular carcinoma (HCC) development in mice. Dietary or genetic obesity induces alterations of gut microbiota, thereby increasing the levels of deoxycholic acid (DCA), a gut bacterial metabolite known to cause DNA damage. The enterohepatic circulation of DCA provokes DNA damage and consequent cellular senescence in hepatic stellate cells (HSCs), which in turn, secretes various inflammatory and tumour-promoting factors in the liver, thus facilitating HCC development in mice after a treatment with DMBA, a chemical carcinogen that causes an oncogenic Ras mutation, at the neonatal stage. Indeed, we observed that obese mice lacking IL-1b gene, an upstream regulator of SASP induction,
show strikingly decreased number of liver tumours. Similar results were also obtained in obese mice depleted senescent HSCs by injection of siRNA against heat shock protein 47. Notably, reducing gut bacteria by treatment of the obese mice with antibiotics resulted in a marked reduction of liver cancer. Furthermore, blocking DCA production by inhibition of microbial 7a-dehydroxylation, the biochemical reaction that produce DCA, efficiently prevents HCC development in obese mice. These results indicate that the DCA-SASP axis in HSCs has key roles in obesity-associated HCC development. Importantly, moreover, signs of cellular senescence and SASP were also observed in the HSCs in the area of HCC arising patients with non-alcoholic steatohepatitis (NASH). In human, emerging evidence has indicated that alterations of intestinal microbiota are associated with obesity. These data, together with the previous observation that high fat consumption resulted in higher faecal DCA concentrations in human, suggest that DCA-induced senescent HSCs may contribute to obesity-associated HCC development via SASP in human as well. These findings provide valuable new insights into the development of obesity-associated cancer and bring with it the promise of better tools for risk assessment, diagnostics and cancer prevention. In this talk, I provide an overview of our work and discuss the next steps, focusing on the potential clinical implications of these findings.

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CROSSTALKS BETWEEN GUT MICROBES AND HOST CELLS CONTROL METABOLISM DURING OBESITY AND DIABETES

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Obesity is associated with a cluster of metabolic disorders, low-grade inflammation, and altered gut microbiota. Over the last 15 years, our work has been devoted to examine how gut microbiota interacts with nutrients and host physiology.

In 2007, we have described the concept of metabolic endotoxemia (increase in plasma LPS levels) as one of the triggering factors leading to the development of metabolic inflammation and insulin resistance. Following this discovery, we found that the major factors involved in the development of these diseases observed upon obesity, diabetes and hepatic steatosis are related to the gut barrier function and gut microbiota composition.

Although the clear mechanisms involved in the bacteria-host interactions are still under investigation, we found that changes in gut microbiota composition are associated with modification of the enteroendocrine functions of the gut at the level of gut peptides (e.g., GLP-1, GLP-2, PYY, ghrelin) and the endocannabinoid system.

More recently, we have proposed that \textit{Akkermansia muciniphila} is a novel key player involved in the control of gut permeability, mucus layer thickness and metabolism during obesity and type 2 diabetes. We also discovered that the most studied probiotic yeast, that is \textit{Saccharomyces boulardii}, plays a key role on obesity and associated metabolic disorders. We found that \textit{S. boulardii}-treated mice exhibited reduced body weight, hepatic steatosis, fat mass and both hepatic and systemic inflammation. Importantly, we also found that \textit{S. boulardii} induced dramatic changes in gut microbial communities at the phylum, family and genus levels.

Finally, our recent data using inducible intestinal epithelial cell specific deletion of a protein from the innate immune system (MyD88) show that the intestinal innate immune system acts as a sensor changing host metabolism according to the nutritional status.

We will discuss the impact of gut microbes on hepatic steatosis, energy expenditure, glucose homeostasis, fat mass and inflammation in this context.

Disclosure of Interest: P. D. Cani: Consultant / Advisor: Biocodex benelux; J&J
NAFLD, CARDIOVASCULAR AND CARDIAC DISEASE

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Patients with NAFLD usually have features of the metabolic syndrome (MetS) and also have a myriad of other emerging cardiovascular (CVD) risk factors (1-3). This finding has important clinical implications for the development of future CVD events among these patients. A recent comprehensive meta-analysis of 27 cross-sectional studies reported a strong association between NAFLD detected by biopsy or imaging and several markers of subclinical atherosclerosis, such as increased carotid intima-media thickness (16 studies), increased coronary artery calcification (7 studies), impaired flow-mediated vasodilation (7 studies) and arterial stiffness (6 studies). All of these associations were independent of classical CVD risk factors and MetS features across a wide range of patient populations (4). To date, there are about 20 retrospective and prospective studies that have assessed the relationship between NAFLD diagnosed on biopsy or imaging and the risk of developing fatal and nonfatal CVD events and most of these studies support the notion that CVD is a serious threat to patients with NAFLD. Additionally, NAFLD is a risk factor for type 2 diabetes(5) and a change in NAFLD status over time is associated changing risk of type 2 diabetes(6) and hypertension(7), two important CVD risk factors. With regard to biopsy-diagnosed NAFLD some retrospective studies with reasonably long follow-up have clearly shown that all-cause, CVD and liver-related mortality were significantly higher in NAFLD patients than in matched control populations. These studies have also shown that the presence and severity of hepatic fibrosis is the main determinant of all-cause and cause-specific mortality, and that CVD is a very common cause of mortality among these patients. With regard to imaging-diagnosed NAFLD several large prospective studies have also consistently shown that NAFLD is associated with an increased risk of fatal and nonfatal CVD events, independently of established CVD risk factors both in individuals with, and without type 2 diabetes. A meta-analysis published in 2011, concluded that patients with NAFLD (diagnosed by histology or imaging) had a twofold higher risk of fatal and nonfatal CVD events (8).
It is now also becoming evident that NAFLD is associated with abnormalities in myocardial metabolism; for a more detailed review see (9) with studies showing that subjects with higher intra-hepatic fat content have higher myocardial fat content. Interestingly, cardiac steatosis is a strong predictor of diastolic dysfunction (10) and similar findings have also been confirmed in paediatric NAFLD. Recently, mildly elevated liver transaminases have been shown to be independently associated with increased incidence of atrial fibrillation (AF) in the Framingham Heart Study cohort (11). More direct evidence of increased risk of AF associated with NAFLD has been recently reported by our group (13, 14). Interestingly, recent data (15) have shown that NAFLD is also independently linked with a prolonged QTc interval, i.e., a powerful predictor of ventricular arrhythmias and sudden cardiac death, which might explain in part the increased CVD mortality associated with NAFLD. Finally, the presence of aortic-valve sclerosis, i.e., a progressive disease that shares multiple pathogenic risk factors with CVD, has also been linked with NAFLD, independently of established CVD risk factors.

This presentation will focus on the rapidly expanding body of clinical evidence that supports the concept of NAFLD as a multisystem disease focussing on cardiovascular and cardiac disease. Potential treatments and their cardiovascular benefits or harms, will also be discussed.

References:
NASH AND HEPATIC/EXTRAHEPATIC CANCER

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NAFLD is associated with increased risk of both hepatocellular carcinoma (HCC) and some (obesity-related) extrahepatic malignancies such as colorectal cancer. NAFLD is a major contributor to the overall health burden associated with HCC. The majority of HCCs in NAFLD develop on a background of cirrhosis but the chronic inflammatory milieu associated with NASH is also likely to drive hepatocarcinogenesis in the absence of cirrhosis. Aetiologically, the contribution of local pro-carcinogenic mechanisms linked to NAFLD, including inflammatory signalling and lipotoxic DNA mutagenesis, versus systemic factors linked to excess body weight, has not been elucidated.

Excess body weight is associated with increased risk of cancer in the luminal gastrointestinal tract, particularly evident in the oesophagus and colorectum. Observational studies suggest a link between visceral adiposity, metabolic health status (specifically metabolic syndrome and its biomarker in observational studies type II diabetes mellitus) and risk of both oesophageal cancer (adenocarcinoma) and colorectal cancer (adenocarcinoma), as well as the respective pre-malignant lesion in each organ (Barrett’s metaplasia and colorectal adenoma). Mechanistically, it is currently accepted that aetiological factors driving NAFLD in individuals with excess body weight, including chronic inflammation and intestinal dysbiosis, are shared by colorectal carcinogenesis. Therefore, it is not surprising that observational data have emerged linking NAFLD (specifically NASH) with increased risk of colorectal adenoma and adenocarcinoma. Large studies exploring the relationship between NAFLD and oesophageal cancer risk in those with Barrett’s oesophagus are awaited.

Data on the effect of NAFLD interventions on subsequent cancer risk are required. Methodological difficulties in retrospective studies include reverse causation and recall bias. Some observational data on intentional weight loss on reduced obesity-related cancer risk are available but existing studies stop short of investigation of co-existent NAFLD. Other interventions such as increased physical activity likely produce dual benefit for NAFLD and extrahepatic (colorectal) cancer risk.
BARIATRIC SURGERY, MODULATION OF MICROBIOTA AND NASH

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Composed of about 10 trillion cells, the human body is host to 100 trillion bacteria which constitute an extremely rich and diverse microbiota. From a genetic point of view there is 500,000 bacterial genes on average compared to 23,000 genes in the human genome. Indeed, a human being is the result of a mutualistic association, stemming from a co-evolution, whom balance is essential to maintaining the health and well-being. The gut microbiota is now seen as a full organ linking (external) environmental factors and biology of the organism (the host). It provides essential functions throughout life. An imbalance of the gut microbiota or dysbiosis has been demonstrated in a variety of human diseases, whether metabolic, cardiovascular or immuno-inflammatory. These observations have been made in particular through the development, in recent years, of tools for the study of the metagenome allowing the sequencing of bacterial genes from the gut microbiota. A factor very frequently found is the loss of bacterial diversity. Although the loss of diversity is typically associated with taking antibiotics, it is also found in other diseases such as cystic fibrosis, intestinal disorders, and more recently in metabolic diseases such as diabetes and obesity. The gut microbiota seems also to be involved in the development of NASH in obese patients. Recently, our team has helped to show that obese people with a loss of bacterial diversity had more risk factors (dyslipidemia, low-grade inflammation) and improved less these risk factors with a restrictive but rich in fibers diet. This presentation will review the recent discoveries in the field and will focus on bariatric surgery effects on gut microbiota and metabolic related phenotypes including NASH.
References:


SERUM METABOLOMICS IN NAFLD

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Mat1a KO mice spontaneously develop nonalcoholic steatohepatitis (NASH), which highlights the role of this gene in hepatic energy stores.

MAT1A encodes the enzyme that catalyses the conversion of methionine into S-adenosylmethionine (SAMe), the main biological methyl donor. NASH patients often have reduced expression of MAT1A, indicating that the Mat1a KO mouse model not only recapitulates the histological and metabolic features of NASH but it is also a relevant model to study human NASH.

Here we used NASH Mat1a KO mice (showing ultrasound fatty liver and elevated ALT) to: 1) evaluate if oral SAMe treatment for two months reverts NASH; 2) study the hepatic metabolic phenotype of NASH in Mat1a KO mice treated with placebo or SAMe (over 500 metabolites were analysed); and 3) obtain a lipid signature of NASH through the serum lipidomic phenotype in Mat1a KO mice. To translate these findings to humans, we examined the presence of this identified lipid signature of NASH in Mat1a KO mouse in the serum of patients with biopsy proven NASH diagnosis.

1) The histological exam of liver samples revealed a marked reduction of steatosis, inflammation, necrosis and fibrosis as well as a normalization of serum ALT after SAMe treatment in Mat1a KO mice. 2) Lipidomic studies show that the main effect of SAMe in NASH treatment is to improve mitochondrial function, which leads to the reestablishment of a normal response to the accumulation of hepatic fatty acids (FA) (increased FA β-oxidation and decreased lipogenesis) and to the reduction of FA and triglyceride content. In addition to this, SAMe reestablished normal bile acid metabolism.3) We compare the serum lipidomic profile in WT and Mat1a KO mice to generate a serum signature of NASH comprised of 50 metabolites (M-type signature). To translate these findings to humans, we carried out a hierarchical clustering of the serum lipidomic data of 134 patients with biopsy-confirmed NASH. Patients were classified into two well-defined clusters based on optimum average silhouette width: one cluster with an M-type signature that included 78 patients (58.2%) and a second cluster that contained 56 patients (41.8%), which suggests that the target for NASH patients with an M-type signature may be considered when assessing the efficacy of SAMe treatment.

These results show that SAMe treatment halted progression of NASH and reverted toward normal histology in Mat1a KO mice.

Disclosure of Interest: J. Mato: Consultant/ Advisor: ABBOTT, Stockholder: OWL
TREATMENT OF NON ALCOHOLIC FATTY LIVER DISEASE: MODULATING THE GUT MICROBIOTA

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A close interplay exists between the gut and liver, named “gut-liver axis” Non Alcoholic Fatty Liver Disease (NAFLD) is currently defined as a multifactorial disease involving the gut microbiota in both the pathogenesis and progression to its more advanced stages. Derangement of the gut microbiota, that means small intestinal bacterial overgrowth or dysbiosis could promote insulin resistance, increase endogenous ethanol production, induce choline deficiency and through an increased gut permeability, induce the passage of bacteria and bacterial products into the bloodstream with a systemic inflammatory state. Therefore, gut microbiota may represent an ideal target of new therapeutic strategies for NAFLD prevention and treatment.

In the last ten years, prebiotics, probiotics, and symbiotics that may modulate the gut microbiota have been tested for the treatment of NAFLD.

In ob/ob mice, modifications of the gut microbiota following probiotic administration improved hepatic insulin sensitivity, hepatic steatosis and inflammation. A systematic review of animal model studies, showed that prebiotic supplementation positively impacts NAFLD by modifying the gut microbiota, reducing body fat, and improving gluoregulation.

Overall both probiotics and prebiotics have shown considerable efficacy, however, the majority of available data, come from animal model studies furnishing only biological plausible health benefits whereas, there are limited clinical studies supporting systematic evidence-based recommendations. Unfortunately, no studies have addressed the effect of antibiotics to treat NAFLD.

In conclusion modulation of the gut microbiota may represent a new way to treat or prevent NAFLD. At present, however, we need randomized controlled human studies involving many patients and a long follow up even if, several unmet needs concerning probiotics and prebiotics persist such as: which is best type, dose and duration and what outcome measures are more appropriate.
NOVEL THERAPIES FOR NASH: WHAT IS ON THE HORIZON?

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NASH is a common liver disease that increases liver-related mortality and reduces survival. The need for optimal management of NASH is therefore a priority for today’s practicing hepatologist. The rationale for specific pharmacological therapy in NASH is based on the potential for disease progression and the difficulties, in many patients, to successfully implement, in the long term, diet and lifestyle changes. Even in those that succeed to do so, limited evidence exists that severe liver injury in NASH can be reversed by diet and lifestyle measures alone, hence the need for pharmacological therapies specifically aimed at improving NASH. The PIVENS trial that compared the efficacy of pioglitazone and of vitamin E vs. placebo resulted in a shift in paradigm because it demonstrated that both an insulin sensitizer with no notable direct hepatic actions and an anti-oxidant hepatoprotectant with no direct effect on insulin resistance can improve histology in NASH. Therefore current trials are testing pharmacological agents with pleiotropic actions. Obethicholic acid, a farnesoid X receptor (FXR) agonist has metabolic as well as hepatoprotective actions. It reduces lipogenesis and increases fatty acid beta oxidation it reduces neoglucogeneis and improves insulin signaling but has also anti-inflammatory and possibly anti-fibrotic effects in the liver, kidney and intestine. A large phase 2b study has demonstrated significant improvement in all histological lesions constitutive of NASH but also of fibrosis. Another prominent candidate, GFT 505, a peroxisome proliferator activated receptor (PPAR) alpha and delta agonist. This oral compound which has an extensive enterohepatic cycle and is liver targeted does not have PPAR gamma activity and therefore is not expected to induce weight gain or be associated with unwanted cardiovascular effects of glitazones. Phase 2a trials in several hundred patients have demonstrated an improvement in hepatic and peripheral insulin sensitivity, in dyslipidemia, in systemic inflammatory markers and in liver enzymes. Importantly, animal studies in both NASH models and in liver fibrosis models have shown an improvement in experimental steatohepatitis but also in fibrosis. A large phase 2 b trial of GFT 505 is currently underway. Another promising candidate is cenicriviroc (CVC), a dual selective inhibitor of ligand binding to C-C chemokine receptor type 2 and type 5 (CCR2 and CCR5). CVC blocks the binding of MCP1 to CCR2 and of RANTES and MIP1α and 1b to CCR5. Therefore CVC decreases recruitment, migration and infiltration of pro-
inflammatory monocytes to the site of liver injury which should relieve hepatic inflammation and also decreases Kupffer cell and hepatic stellate cells activation and migration which should trigger anti-fibrotic effects. Whether CVC also has effects on adipose tissue insulin resistance through the modulation of adipose tissue inflammation, remains to be determined. A large phase 2b trial of CVC is underway in patients with histologically defined NASH. Other approaches are directed towards inhibiting hepatic lipogenesis through the inhibition of different enzymes that regulate de novo lipogenesis. One such candidate is aramchol, a fatty acid (arachidic acid)-bile acid (cholic acid) conjugate that has strong antisteatogenic effects in the rat and is able to reduce the hepatic triglyceride content in humans. A large phase 2b trial testing Aramchol vs. placebo is currently underway. While some of these compounds might have antifibrotic effects, they are, for the most part, directed against steatohepatitis. A totally different approach would be to specifically test antifibrotic agents in trials with fibrotic end-points. Simtuzumab is a humanized monoclonal antibody that is directed against lysyl oxidase-like 2 (LOXL2) an enzyme that drives cross-linking of collagen fibers and that is key to progression of fibrosis in the human liver. Immunohistochemical studies have shown increased expression of LOXL2 in human liver fibrosis, both HCV and NASH related. Very large phase 3 trials are currently testing the parenteral administration of simtuzumab in NASH patients, both cirrhotic and non-cirrhotic. If all or some of these anti-NASH or antifibrotic drugs are effective, it might be ultimately possible to devise a personalized, tailored therapy in patients with NASH in order to avoid disease progression and the occurrence of cirrhosis.

Disclosure of Interest: V. Ratziu Consultant/ Advisor: Intercept, Genfit, Galmed, Tobira
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POSTERS
ABSTRACTS
SERUM BILE ACID LEVELS IN CHILDREN AND ADOLESCENTS WITH NONALCOHOLIC FATTY LIVER DISEASE: A MARKER FOR PROGRESSION?

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is frequent in obese children. A reliable non-invasive biomarker for monitoring of progression to liver fibrosis would be useful. Serum bile acid (BA) levels are elevated in cirrhosis, probably for mechanical reasons. Interestingly, BA can influence glucose and lipid metabolism by stimulating insulin release via the TGR5/GLP1 pathway; and, reciprocally, insulin can downregulate BA synthesis from cholesterol via the FXR/SHP and/or the PI3K/AKT pathways. We hypothesized that changes in BA levels in NAFLD vary depending on grade of fibrosis.

Material and Methods: In this multicenter study adolescents with NAFLD (n=92) were classified between stages of fibrosis (non-fibrosis n=27; fibrosis ≥1 n=65) based on liver-biopsy findings. Metabolic and cholestatic status was assessed by blood tests (glucose, insulin, cholesterol, LDL, HDL, AST, bilirubin, ALT, GGT). The full BA pool, including 15 BA species, was measured by HPLC-MS/MS and compared to healthy controls (n=105).

Results: Both groups showed hyperglycemia (non-fibrosis 126±44; fibrosis 119±18 mg/dl), hyperinsulinism (83±33 vs 88±41 μE/ml), and elevated ALT levels (63±20 vs 87±58 U/l). Non-fibrotic adolescents had significantly (p<0.001) decreased median BA levels (1.28; range 1.18 – 2.34 μmol/l) compared to controls (3.36; range 2.16 – 4.69 μmol/l). In fibrosis BA values increased (1.86; 1.05 – 3.22 μmol/l; p<0.001). Non-fibrotic patients lacked glycine-conjugated BA with a significant (p<0.05) predominance of unconjugated BA. In fibrosis, glycine-conjugated BA values rose and the BA pool resembled that in healthy controls. Other values did not differ significantly between the groups.

Conclusions: BA levels decrease in early NAFLD and seem to increase continuously during progression to fibrosis and cirrhosis. BA may serve as a non-invasive biomarker for progression of disease.
P02

CURCUMIN, ANTI-OXIDANT, AND PIOGLITAZONE THERAPY WITH INCLUSION OF VITAMIN E IN NON ALCOHOLIC FATTY LIVER DISEASE-A RANDOMIZED OPEN LABEL PLACEBO CONTROLLED CLINICAL PROSPECTIVE TRIAL (CAPTIVE)

P Patrick Basu* 1, 2, Niraj J. Shah 3, Robert S. Brown Jr 4
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Introduction: NAFLD is a global clinical challenge which progresses to cirrhosis and liver cancer. Defective transport of free fatty acids and mitochondrial dysfunction lead to explosion of a series of free radicals, apoptosis, up regulated cytokines and fibrogenesis ultimately causing cirrhosis and cancer. Curcumin is a pan-antioxidant with anti-inflammatory, anti-apoptotic, anti-microbial, and anti-fibrogenic properties.

Aims: This study evaluates the role of curcumin in NAFLD to progression of NASH.

Material and Methods: Eighty patients (n=80) with mean BMI 29% were recruited, NAFLD score 0.66, NASH fibrotic score 0.33, HOMA IR 3.8, ALT 58, LDLc 143, HDLc 29, Triglyceride 186 and Adipokines (Leptin, Adiponectin, Retinal Binding Proteins) were divided into Group A- (n=20) Pioglitazone 15mg, Group B- (n=20) vitamin E, Group C- (n=20) curcumin (all the three above groups received placebo), and Group D (n=20) vitamin E plus curcumin. Pre and post values (Triglycerides, LDLc, HDLc, ALT, HOMA-IR, TNF-alfa, Leptin, Adiponectin, Retinol Binding Protein, HBA1c, Serum necro-inflammatory NAFLD and NASH fibrotic score were analysed at 3, 6, and 12 months. Diet and exercise were left unchanged. Daily alcohol content was less than 30 grams. Exclusion; HIV, Medications causing fatty liver including herbal supplements, Lipodystropy, Overt diabetes mellitus, Pregnancy. hypersensitivity to study medications.
**Results:** Group A- Minimal changes on ALT, HbA1c, HOMA, lipids, no changes in TNF-alfa, adipokines, lipid profile and necro-inflammatory score and/or NASH fibrosis score. Group B and Group C had modest changes in ALT, lipid profile, HbA1c and HOMA; while no changes in adipokines, necro-inflammatory score and fibrotic score. Group D had significant changes in all scores particularly the adipokines and small improvements in fibrotic score. All patients tolerated the medications well.

**Conclusions:** This study postulates the positive effects of Curcumin added to vitamin E in NAFLD subgroups; even preventing NASH with a modest anti-fibrotic effect and improved necroinflammatory score; and impressive changes in adipokines levels. Additive effects of Curcumin with vitamin E has significant effects on serum lipids and insulin sensitivity. Unavailability of Pre and post liver biopsy was the limitation of this study. A large control trial needs to validate.
BERBERINE WITH ALFA LIPOIC ACID (ALA) IN NON ALCOHOLIC STEATO-HEPATITIS (NASH). A RANDOMIZED DOUBLE BLINDED PLACEBO CONTROL TRIAL. A CLINICAL PILOT—THE BANISH TRIAL

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Introduction: NASH is a globally challenging clinical morbidly; causing cirrhosis and liver cancer in due time with formidable cost burden 1,2. Therapeutic modalities have not yet been fully established 1,2. ALA attenuates steatohepatitis through inhibition of several pathways— the intra hepatic mechanism of fatty acid transport, lipid per oxidation, free radical production and apoptosis 3. Berberine is a natural substance extracted from plants like Berberis which is found to up-regulate intra hepatic pathways as insulin sensitizer, via GLP-1 up regulation and Acyl palmotyl mechanism on fatty acid oxidation, induction of PPAR gama; all that blocks the terminal inflammatory Cytokine release TNF Alfa to preclude fibrosis, cirrhosis and HCC 4.

Aims: To evaluate the role of Berberine with ALA in NASH in normalizing ALT (primary endpoint) and accessing response in liver inflammatory markers (secondary endpoints)

Material and Methods: Hundred and twenty patients (n=120) with NASH were recruited. Mean BMI 29.9% (29% to 32%) with 69 males and 51 females. Mean HbA1C 6.2 (5.9-6.8), Mean HOMA 2.7 (2.1-3.6), ALT 54 (38-79), Triglyceride 287 (233-344), LDL c 163 (129-176), Leptin 63 (43-98), Adiponectin 0.9 (0.1-1.1), RBP 4 of 5.8 (4.0-6.8), TNF Alfa 3.8 (2.1-4.8), IL 12 of 5.3 (3.9-7.8), Serum Fibrotic and Steatotic scores were measured at 0 and then at 6 months. Daily allowed caloric content was 2,000 cal/ day, no documented exercise except daily activities at baseline for 6 months.

Exclusion Criteria: Chronic hepatitis B and C, HIV, Other liver diseases, Alcohol consumption more than 30 grams a week, Diabetes, Drugs (Steroids, Tamoxifen) causing fatty liver or any lipodystrophic syndromes

Conclusions: All the study arms showed statistically significant (p<0.05) post interventional change except for Adiponectin in group A (p=0.09)

References
THE IMPACT OF GANODERMA LUCIDUM WATER EXTRACTS ON GUT MICROBIOTA AND METABOLIC SYNDROME IN HIGH FAT DIET-INDUCED OBESE MICE

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Introduction: Gut microbiota plays a pivotal role in controlling energy metabolism, intestinal homeostasis and systemic immunity in host. Alternation of gut microbial composition has been well characterized to be highly correlated with chronic systemic inflammation and metabolic-related diseases. These include obesity-induced metabolic abnormalities that elevate individual’s risks of developing insulin-resistance and type II diabetes, and nonalcoholic fatty liver disease (NAFLD). Traditional Chinese herbal medicines (TCM) have been used for more than two thousand years and prove benefit to host health by immuno-modulation. However, the impact and mechanism of TCM on gut microbiota and obesity-associated metabolic diseases and NAFLD remain not clear.

Aims: Here, we study the effect of water extract of Ganoderma lucidum (WEGL) on preventive effect of obesity related metabolic syndrome and NAFLD, and see their relationship to the gut microbiota.

Material and Methods: The Ganoderma lucidum strain initially selected and characterized at Chang Gung Biotechnology (Taipei, Taiwan) and the water extract of cultured mycelium. Mice was fed following either chow diet or HFD for 12 weeks, as well as daily oral administration of 100 μl of either saline for chow, or WEGL at 4%, or 8% (w/v) for HFD-fed mice. In this study, we not only measured the body weight, tissue weight, liver function and fat accumulation, but also detected the inflammatory cytokines and insulin resistant index.

Results: Daily oral administration of WEGL for 12 weeks exerts significant loss of body weight, and liver and visceral adipose tissue weight in HFD-induced obese mice. Furthermore, Triacylglycerol accumulation and immune cells infiltration were also alleviated. Besides, obesity-associates insulin resistance was also significantly improved by WEGL treatment. Of note, the high abundance of lipopolysaccharide (LPS) measured in
HFD mice and positively correlated with TG accumulation, TNF-α secretion level and HOMA-IR index was also reversed by WEGL administration. The microbiota structure was also modulated by WEGL treatment.

**Conclusions:** Currently, WEGL may potentially function as a potential herbal prebiotic whose function may involve gut microbiota, resulting in gut homeostasis and reduced inflammation in HFD-derived metabolic syndrome in mice.
YI - P05

CHARACTERISTICS OF 665 PATIENTS WITH BLEEDED ESOPHAGEAL VARICES AS A GUIDE FOR TRANSPLANT CENTERS: THE DAY IS TODAY FOR NAFLD!

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Introduction: The most common cause of chronic liver disease is NAFLD in developed countries. It is likely to emerged as the most common cause of liver transplantation and liver related death in the next decade.

Aims: Our aim was to evaluate the charasteristics of bleeded esophageal varices at a tertiary referral hospital as a single centre experience.

Material and Methods: We evaluated our Endoscopy Unit records from 2003 to 2014. We had the database of 665 patients with bleeded esophageal varices and treated, accordingly. We differed the patients into 3 groups as hepatitis B, hepatitis C, and nonB/nonC. Any type of esophageal varices, not due to hep B and hep C was defined as nonB/nonC (due to NAFLD, alcohol etc.). The groups were evaluated by the existence of fundal varices, liver mass detected by transabdominal ultrasound (nodule >10 mm and further examination showed HCC), portal vein thrombosis (PVT), cirrhosis, mortality and by the age and gender.

Results: Of the 665 patients with bleeded esophageal varices, 258 patients had good data and further analysed. Table 1 shows the characteristics of the patients. Mortality rates and the existence of HCC were not changed by the occurrence of PVT.

Conclusions: The cause of bleeded esophageal varices was NAFLD in up to half of the patients in this study. The occurence of HCC in viral group differed from the non-viral group, remarkably. Patients with hep C were older and had a higher rate of liver mass than others. Mortality rate among the groups was not changed by the etiology. Our results showed a proportionally greater rise in liver transplant candidates due to NAFLD. These findings would be good guide for evaluating the patients at the transplant center and taking preventive measures by the health policy makers.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>NonHacOC</th>
<th>HBV</th>
<th>HCV</th>
<th>The study group</th>
<th>P (statistically significance &lt; 0.05)</th>
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<td>57.7±13.3 (24-90)</td>
<td>62.9±12.2 (28-79)</td>
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<td>84%</td>
<td>87%</td>
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<td>Mortality</td>
<td>42%</td>
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GALECTIN-3 DEFICIENCY EXACERBATES LIVER STEATOSIS BUT PROTECTS FROM STEATOHEPATITIS AND IL-33/IL-13 DEPENDENT FIBROSIS IN HFD-INDUCED OBESITY MOUSE MODEL

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Introduction: Gal-3 participates in the pathogenesis of metabolic disorders, but its importance in obesity-associated liver pathology is incompletely defined.

Aims: In this study, we aimed to dissect the role of Gal-3 in liver inflammatory response and fibrosis, key events in the pathogenesis and progression of nonalcoholic steatohepatitis (NASH) induced by obesogenic high-fat diet (HFD).

Material and Methods: Gal-3-deficient (LGALS3−/−) and wild-type (LGALS3+/-) C57Bl/6 mice received HFD (60% kcal fat) or standard chow diet for 24 weeks and metabolic parameters, gene expression and immunophenotypic analyses were performed.

Results: Compared to WT mice, HFD-fed LGALS3−/− mice developed, in addition to increased obesity and type 2 diabetes, more pronounced liver steatosis accompanied with increased hepatic PPAR-γ and Cd36 expression. However, ALT and AST levels, liver injury, inflammation and fibrosis scores, and hepatic procollagen and α-SMA mRNA expression were significantly lower in obese LGALS3−/− mice. In addition, livers of obese LGALS3−/− mice contained lower proportions of mature myeloid DCs, proinflammatory monocytes (CD11b+Ly6C+) and M1-macrophages (F4/80+CD11c+CD206−) and lower CCL2 chemokine, NLRP3 inflammasome and IL-1β mRNA expression compared to diet-matched WT mice. Furthermore, profibrogenic IL-33 and IL-13 in liver homogenates and hepatic IL-33 receptor (IL-33R) and IL-13 mRNA expression were lower in LGALS3−/− mice than in WT mice, while hepatic TGF-β levels were similar. Moreover, in contrast to WT macrophages, LGALS3−/− peritoneal macrophages failed to upregulate IL-33R expression and IL-13 production in vitro in response to stimulation with recombinant mouse IL-33.

Conclusions: Gal-3 ablation promotes steatosis, but prevents liver injury, inflammation and fibrosis in obesogenic model of NASH by attenuating recruitment of proinflammatory myeloid cells and profibrogenic IL-33 and IL-13 in liver.
ROLE OF FGF21 IN NON-ALCOHOLIC FATTY LIVER DISEASE PROGRESSION

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Introduction: FGF21 plays a central role in glucose and lipid homeostasis in humans.

Aims: The main aim of this study was to elucidate the role of FGF21 on NAFLD, analysing FGF21 gene expression in the liver, serum FGF21 levels and genotypes from SNP rs838145 in FGF21 gene located at chromosome 19.

Material and Methods: This study was performed in three phases:
1. Evaluation of liver FGF21 gene expression from liver biopsy samples by RT-PCR (n=20, 10/20 NASH and 10/20 simple steatosis), evaluated by mean fold change. It was also evaluated PPAR-γ, upregulated by FGF21.
2. FGF21 levels were measured in twelve hours-fasting serum from 60 patients using a commercial ELISA kit (Abnova, USA).
3. Single-nucleotide polymorphism (SNP) in FGF21 gene (rs838145) was analysed by Taqman assay (n=240; mean age 47.01±13.16; 41% men). This SNP was also calculated in 178 healthy controls. Liver damage was assessed using Kleiner score, evaluating lobular inflammation, ballooning and steatosis degree. Statistical analysis was performed using SSPS v22.
Results:
1. FGF21 gene expression was found up-regulated in NASH (mean fold change 4.37+4.09) but down-regulated in simple steatosis (mean fold change -8.59+ 7.11). No differences were seen based on diabetes or dyslipidemia. PPAR-γ was found upregulated in NASH (mean fold change 2.05+0.62).
2. ELISA FGF21 serum levels were significantly associated with fibrosis stages (r=0.48, n=60, p<0.0001).
3. Genotypic frequency was similar in healthy controls and NAFLD patients: genotype GG [30/178(16.9%) vs. 41/237(17.3%)], genotype AA [75/178(42.1%) vs. 87/237 (36.7%)] and genotype AG [73/178(41%) vs. 109/237(46%)];p=ns. Genotype GG was found associated with NASH (O.R.: 2.43 (95%CI 1.110-5.357, p=0.026) and advanced fibrosis (O.R.:3.39 (95%CI 1.369-8.403, p=0.008).

Conclusions: Intrahepatic FGF21 gene expression was enhanced in NASH. FGF21 serum levels were correlated to fibrosis stages. GG genotype was associated with increased risk of suffering NASH and advanced fibrosis. Due to FGF21 plays a key role in carbohydrate metabolism regulation, FGF21 resistance could explain the association between elevated levels and disease progression, and could exert a new role on NASH pathogenesis.
NON-INVASIVE METHODS TO DETECT ADVANCED LIVER FIBROSIS IN DIABETIC PATIENTS

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Introduction: Non-alcoholic fat liver disease (NAFLD) is the most common liver disease worldwide. It is associated with diabetes mellitus and can progress to end stage liver disease.

Aims: The aim was to determinate which non-invasive method among LSM (Transient Elastography – TE), AST/ALT, APRI (AST (/ULN)/Platelet x 100) and FIB-4 score could have the better performance for prediction of advanced liver fibrosis (≥F3 by NASH CRN Scoring System) in diabetics NAFLD patients.

Material and Methods: Diabetics patients from a tertiary hospital in Rio de Janeiro/Brazil with biopsy proven NAFLD were submitted to TE (M probe) and biochemical analysis to calculate AST/ALT, APRI and FIB-4 score. Continuous variables were compared with the Student’s t test. Correlation was evaluated by the Spearman correlation coefficient. The area under the ROC curves assessed the diagnostic value for advanced fibrosis. A 2-sided P value of less than 0.05 was considered statistically significant. Youden index was used to determinate the better cut-off of the methods.

Results: A total of 64 patients were included, 80% were females. The mean age was 55 ± 7 years old, BMI 30.3 ± 3.7 Kg/m2, abdominal circumference (AC) 102 ± 10cm and mean time between biopsy and TE was 3.7 ± 5.6 months. In 12 patients (19%), the LSM by TE was not valid with 1 advanced fibrosis patient in this group. AC was the factor related to a not valid LSM (p<0.05). Advanced fibrosis was present in 5 patients (8%). The diagnostic accuracy for advanced fibrosis was excellent for LSM (analysis with valid measures, 52 patients) and APRI but bad for FIB-4 and AST/ALT (AUROC: 0.98, 0.92, 0.49 and 0.37, respectively). The optimal cut-off for a diagnostic of advanced fibrosis was 13.9 kPa for LSM (sensitivity of 100%, specificity of 97.9%, PPV of 80% and NPV of 100%) and 0.45 for APRI (sensitivity of 100%, specificity of 88.1%, PPV of 41.7% and NPV of 100%).
The correlation with advanced fibrosis for these cut-offs were 0.88 (p<0.05) and 0.60 (p<0.05) for LSM and APRI, respectively.

**Conclusions:** Measured by TE, LSM seems to be a good non-invasive method, for prediction of advanced liver fibrosis in diabetics NAFLD patients. APRI seems to be also a good method, especially when the measure of LSM by TE is not valid. APRI could be a good choice when TE is not available.

![Box whiskers showing TE and APRI well discriminate the patients with advanced fibrosis from others in the diabetics NAFLD population.](image)
CHOLECYSTECTOMY IS A RISK FACTOR FOR FATTY LIVER DISEASE

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Introduction: Risk factors for fatty liver disease are not well defined, cholecystectomy was considered as a risk factor for fatty liver disease recently.

Aims: To evaluate the association of gallstones and cholecystectomy with fatty liver disease in a regional study.

Material and Methods: Among adult patients admitted in the First Hospital of Jilin University during 2013-2014, gallstone disease and fatty liver disease were diagnosed by abdominal computerized tomography scan or magnetic resonance imaging. Odds ratios (ORs) for the association of gallstone disease with fatty liver disease were calculated using logistic regression analysis to adjust for common associated factors.

Results: Among 59,765 adult patients, the prevalence of gallstones, cholecystectomy and fatty liver disease was 12.7%, 5.4% and 13.0%, respectively. Patients with cholecystectomy had lower age-sex-adjusted prevalence of fatty liver disease (8.6%) than those with gallstones (14.0%) or without gallstone disease (13.2%) (P < 0.01 for all comparisons). Controlling for numerous factors associated with both fatty liver disease and gallstone disease, multivariate-adjusted analysis confirmed the association of fatty liver disease with cholecystectomy (OR = 1.452; 95% confidence interval (CI): 1.3-1.7), but not with gallstones (OR = 1.0; 95% CI: 0.9-1.1).

Conclusions: The association of fatty liver disease with gallstone disease indicates that cholecystectomy itself may be a risk factor for fatty liver disease.
SERUM LEVELS OF SOLUBLE ADVANCED GLYCATION-END PRODUCTS RECEPTOR ARE INVERSELY RELATED WITH LIVER FAT IN NAFLD PATIENTS

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Introduction: The soluble receptor for advanced glycation end products (sRAGE) has been reported to exert protective effects against diabetes and cardiovascular diseases. Aims: In this study, we aimed to identify if sRAGE levels may be associated with liver fat in patients with non-alcoholic fatty liver disease (NAFLD).

Material and Methods: sRAGE levels were analyzed by ELISA in a cross sectional analysis of NAFLD patients (n=55). Hepatic ultrasound, fasting blood tests and anthropometrics were assessed. Liver fat was quantified by the hepatorenal-ultrasound index (HRI) representing the ratio between the brightness level of the liver and the right kidney (≥1.5 indicates NAFLD). A multivariate logistic regression analysis was performed to test the adjusted association between sRAGE levels and HRI.

Results: The mean serum levels of sRAGE were lower (1042.83±286.65 vs. 1377.90±506.45 ng/L, P=0.005) among subjects with higher level of steatosis (classified according to HRI above the median >2.07 score, indicating on liver fat > 25%). Serum sRAGE levels were inversely correlated with HRI (r= -0.40, P=0.003) and the percent of patients with HRI above the median decreased in a dose-response relationship by sRAGE tertiles (72.2%, 57.9% and 22.2%, P for trend=0.004). sRAGE serum levels were also inversely correlated with serum uric acid (r= -0.30, P=0.026), BMI (r= -0.39, P=0.003) and waist circumference (r= -0.30, P=0.025). In a multivariate analysis, sRAGE independently predicted a higher level of steatosis with adjustment for age, gender, BMI and fasting serum insulin [for sRAGE as continuous (OR=0.998, 0.996-0.999 95% CI, P=0.018) and as upper-tertile (OR=0.169, 0.040-0.715 95% CI, P=0.016)].

Conclusions: sRAGE is inversely and independently associated with liver fat in NAFLD and may serve as a potential biomarker. Its role in the pathogenesis of NAFLD remains to be elucidated.
QUERCETIN EFFECTS ON LIPID DROPLETS: THE ROLE OF PNPL3 GENOTYPE IN NAFLD IN VITRO MODEL.

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Aims: To study the effect of quercetin on lipid droplets size in an in vitro model of non-alcoholic fatty liver disease, influence of PNPLA3 genotype.

Material and Methods: Human Huh7.5 and HepG2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine (all from Invitrogen), 100 U/mL penicillin and 100 U/mL streptomycin in a humidified atmosphere at 37ºC and with 5% CO2. Cells were exposed to oleic acid (1mM) for 48 hours and treated with quercetin 50μM. Images were acquired with an epifluorescence microscope and were analysed with software (Imaging Software cell^F). Cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilised with 0.3% Triton X-100 for 2 minutes. Nuclei were stained with 40,6-diamidino-2-phenylindole (DAPI), and neutral lipids were stained with Oil Red O (ORO). PNPLA3 (rs738409) genotype was determined by RT-PCR Taqman probes.

Results: Both cells were GG-PNPLA3 genotype. LDs size (area) increased significantly by oleic acid 1mM (HepG2: fold induction: 8.22±4.62 and Huh7.5: 9.58±9.2), not found differences between theses (p:0.72). After 48h of quercetin 50 uM LD size significantly decreased in both kind of cells with or without oleic acid: area (μm2): HepG2 1.25±0.29; HepG2+Q50μM: 0.72±0.75; HepG2+AO1mM: 8.22±4.62; HepG2+AO1mM+Q50μM: 2.15±1.15 and Huh7.5 PNPLA3-GG: area (μm2): Huh7.5: 1.08±0.64; Huh7.5+Q50μM:0.63±0.35; Huh7.5+AO1mM: 9.58±9.2; Huh7.5+AO1mM+Q50μM: 1.90±1.4, Fig-1.

Conclusions: Quercetin modifies LDs size in cells without oleic acid 1mM and in NAFLD in vitro model. This flavonoid may interfere in the development of NAFLD in presence of unfavourable genotype GG of PNPLA3.
EFFECTS OF URSODEOXYCHOLIC ACID AND PIOGLITAZONE LONG THERAPY ON HEPATOCYTES CHANGES IN NASH PATIENTS

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Introduction: NASH can progress to cirrhosis and hepatocellular carcinoma.

Aims: The aim of this study was to investigate the effects of ursodeoxycholic acid (UDCA) and pioglitazone long therapy on electronomicroscopic, morphometrical and cytogenetic changes of hepatocytes and insulin resistance (IR) in NASH patients.

Material and Methods: In 27 NASH patients [age, 47.3±6.9 yr; body mass index (BMI), 33.9±3.5 kg/m²] and 12 healthy volunteers [age, 44.3±5.5 yr; BMI, 24.2±2.8 kg/m²] obesity and IR were determined by BMI and HOMA-IR. Circulating insulin levels were measured by the immuno-assay method. Electronomicroscopic, morphometrical, cytogenetic examinations of hepatocytes and hepatocyte nuclei were investigated. 15 NASH patients were treated with 10-15 mg/kg/day of UDCA (group I), 12 NASH patients were treated with 10-15 mg/kg/day of UDCA+ pioglitazone 15-30 mg/day for two years (group II).

Results: After therapy, the condensed chromatin content was decreased in hepatocyte nuclei in group II (p<0.05). The quantity of the pathologically changed nuclei was decreased in hepatocytes more than 2.6 times and 1.3 times (p<0.05) in groups I and II vs. patients before treatment. In group II the area of the hepatocytes profile (AHP) was increased – up to (176.40 ± 13.08) µm² vs. (128.73 ± 11.74) µm² (p=0.01) before treatment and vs. (182.17 ± 2.54) µm² (p>0.05) in control; in group I – (138.84 ± 11.76) µm² vs. (129.25 ± 11.03) µm² (p>0.05) before treatment and vs. (182.17 ± 2.54) µm² (p=0.03) in control. After therapy, one patient (8.33%) in group II and eight (53.33%) in group I had an AHP from 50.0 to 150.0 µm² vs. 10 (83.3%) and 12 (80.0%) patients accordingly before treatment; in healthy volunteers AHP ranged from 100.0 to 250.0 µm² (85.42%). The area of the nuclear profile of NASH patients in group II was increased – up to (32.45 ± 2.89) µm² vs. (21.52 ± 2.17 µm²) (p=0.03) and vs. (35.32 ± 0.60) µm² in control (p>0.05), in group I – (25.86 ± 2.45) µm² vs. (21.83 ± 2.15 µm²) (p>0.05) before treatment and vs. (35.32 ± 0.60) µm² in control (p=0.04). Compared with group I, HOMA-IR increased in group II after UDCA + pioglitazone therapy (p<0.02).

Conclusions: The UDCA+pioglitazone combination in long-term use for two years has metabolic effects with beneficial cytoprotective properties and improves electronomicroscopic, morphometrical and cytogenetic markers of the hepatocytes and hepatocyte nuclei in NASH patients.
THE DIET AND MICROBIOTA DEPENDENT METABOLITE TMAO IS ASSOCIATED WITH THE SEVERITY OF LIVER DISEASE IN PRIMARY SCLEROSING CHOLANGITIS

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Introduction: Trimethylamine N-oxide (TMAO) is produced in the liver from trimethylamine, which is exclusively generated by gut bacteria from dietary choline and carnitine found in e.g. red meat and dairy products. TMAO may influence bile acids, metabolism and potentially inflammation.

Aims: Given the production in the liver, links to the gut microbiota and bile acid homeostasis, we aimed to investigate the regulation of TMAO in primary sclerosing cholangitis (PSC).

Material and Methods: We measured serum TMAO in 305 PSC patients, 90 ulcerative colitis (UC) patients without PSC and 99 healthy controls (HC), with a median age (male %) of 41 (76), 38 (51) and 40 (60), respectively. Reduced liver function was defined by increased prothrombin time (International Normalized Ratio >1.2 or Normotest <70).

Results: TMAO was lower in PSC patients than in UC and HC (medians 3.1, 4.0 and 3.5, respectively, p<0.05). We hypothesized that this could be explained by reduced liver function affecting TMAO production. Indeed, TMAO in PSC with reduced liver function, but not in those with normal liver function, was lower than in HC (p<0.05). The TMAO level in UC was higher than in PSC irrespective of liver function in the PSC group (p<0.05),
while TMAO was similar in PSC patients with and without inflammatory bowel disease. There was no detectable association between TMAO levels and genetic variants in the region encoding the flavin-containing monooxygenase genes (responsible for converting TMA to TMAO in the liver). Focusing on PSC patients with normal liver function, TMAO was higher in patients reaching a clinical end-point (death or liver transplantation, n=111) during follow-up than those without (median 4.6 vs 2.7, p=0.001). AUROC-analysis yielded an AUC of 0.64 (p<0.001) with an optimal TMAO cut-off of 4.1 µM. PSC patients with TMAO >4.1 µM exhibited shorter transplantation-free survival than patients with TMAO <4.1 µM (p<0.001, Figure 1). In Cox regressions, TMAO >4.1 µM and Mayo risk score were independently associated with transplantation-free survival, with HR 2.0 (95%CI 1.2-3.1, p=0.005) and 1.5 (95%CI 1.2-1.8, p<0.001).

Conclusions: The TMAO levels were decreased in PSC patients with impaired liver function, suggesting that TMAO and associated phenotypes could be influenced by reduced liver function. In PSC patients with normal liver function, elevated TMAO was associated with a shorter liver transplantation-free survival, suggesting that dietary factors and gut microbiota profile may be relevant for the disease prognosis.
EXERCISE REDUCES LIVER FAT BUT NOT CIRCULATING INFLAMMATORY MARKERS OR VISCERAL FAT IN NON-ALCOHOLIC STEATOHEPATITIS

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Introduction: Non-alcoholic steatohepatitis (NASH) represents steatosis and ballooning degeneration, inflammation and fibrosis. Current treatments for NASH are limited, with no studies to date reporting the effects of exercise in people with NASH. Studies in NAFLD show promising effects on liver lipid but the effect on inflammation and visceral fat, key mediators of fibrosis, are not known.

Aims: Determine the effect of a 12-week exercise intervention on liver lipid and circulatory inflammation in people with NASH.

Material and Methods: 24 participants (mean age 52 ± 14 years, BMI 33 ± 6) with histologically characterised NASH (NAFLD Activity Score ≥ 5) received either: resistance exercise (n = 12) or continue standard care (n = 12) over 12 weeks and maintained baseline weight. Participants were not undergoing insulin sensitising treatment, dietary change or regular activity. Subjects with heart/kidney disease or in vivo ferrous material were excluded. Liver lipid content, subcutaneous and visceral adiposity were assessed using magnetic resonance techniques, inflammation, fibrosis markers, insulin sensitivity were assessed at baseline and at 12 weeks.

Results: Resistance exercise produced a significant reduction in liver lipid content (-13 ± 24 vs. 6 ± 15%, P<0.05). There was no effect of exercise on circulatory inflammation (IL6: 1.4 ± 0.8pg/mL to 1.7 ± 1.7pg/mL vs. 2.4 ± 4.6pg/mL to 2.1 ± 2.9pg/mL; TNFα: 2.2 ± 0.6pg/mL to 2.4 ± 0.8pg/mL vs. 2.3 ± 0.7pg/mL to 2.3 ± 0.8pg/mL, P>0.05) or abdominal adiposity (VAT: 187 ± 54cm² to 170 ± 50cm² vs. 170 ± 72cm² to 211 ± 112cm²; SAT: 337 ± 181cm² to 317 ± 158cm² vs. 389 ± 121cm² to 394 ± 112cm², P>0.05).
Metabolically, there was no effect of exercise on HbA1c (51 ± 14mmol/mol to 49 ± 12mmol/mol vs. 46 ± 11mmol/mol to 49 ± 12mmol/mol, P>0.05), HOMA IR (2.3 ± 1.4 to 1.9 ± 0.8 vs. 1.9 ± 1.1 to 1.7 ± 1.1, P>0.05) or 2hour glucose levels or liver enzymes (ALT: 53 ± 25U/L to 52 ± 18U/L vs. 81 ± 59U/L to 71 ± 52U/L; AST: 41 ± 14U/L to 45 ± 12U/L vs. 59 ± 41U/L to 58 ± 45U/L, P>0.05).

**Conclusions:** This is the first study reporting the effects of exercise on liver lipid, abdominal adiposity and circulating inflammation in patients with histologically defined NASH. Exercise produces a significant reduction in liver lipid but no effect on body weight, abdominal adiposity, or circulatory markers of inflammation. These results suggest that exercise alone may be insufficient to target the mediators of NASH and warrants further exploration.
ALGINATE ENRICHED BREAD ATTENUATE CIRCULATING LIPIDS AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction: Non-alcoholic fatty liver disease (NAFLD) affects up to one in three people of developed nations and is considered by some to be the hepatic manifestation of the metabolic syndrome. People with NAFLD have a high level of fat intake which may cause; 1) high energy intake, 2) insulin resistance, and 3) hepatic de novo lipogenesis. Therapeutic interventions for NAFLD are limited. Alginates are polysaccharides extracted from brown algae that are un-digestible in the upper gastrointestinal tract (GI). Specific alginates are able to inhibit the activity of pancreatic lipase and thus reduce fat digestion and absorption. The effect of alginates based in food upon fat absorption is not known, despite the therapeutic implications.

Aims: To determine if alginate enriched bread inhibits fat digestion and circulatory lipids

Material and Methods: Twenty-nine ileostomy patients were fasted overnight and then fed either 100g alginate enriched bread (4% w/w wet dough) with 20g of butter, or 100g of control bread with 20g of butter a month apart in a double blind randomised cross over study. Both alginate and control bread were produced by Greggs Plc master baker. Effluent samples, blood samples, and wellbeing questionnaires were taken at baseline and then every 30 minutes for a total of 5 hours. Ileostomy patients were used to create a model to study fat absorption in the upper GI in isolation.

Results: The alginate bread produced a 20% increase in effluent weight at 300 minutes (2489g vs. 2010g, P<0.05), a 31% increase in total effluent fat (376g vs. 264g, P<0.05) and a significant correlation between fat content effluent weight (r = 0.98, P<0.05). A small reduction in plasma triglycerides was reported when consuming alginate bread, however this was not significant (P>0.05). There were no substantial differences in palatability between the two breads, apart from time point zero where subjects reported an increase in thirst (1.5 ± 1.5 vs. 0.5 ± 0.5, P<0.05) and reduced fullness (2.2 ± 1.8 vs. 1.6 ± 1.6, P<0.05) following consumption of alginate bread.

Conclusions: This is the first study to show that alginate enriched products reduce fat digestion in man. The data shows that alginate enriched products are able to attenuate fat digestion by up to 31%. Alginate enriched products hold potential as a therapeutic weight and metabolic management therapy, without side effects of other known pharmaceutical agents, and should be considered in NAFLD.
LOVASTATIN’S AND PENTOXIFYLLINE’S EFFECTS IN PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS

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Introduction: Today, there is no ideal treatment for nonalcoholic steatohepatitis (NASH).

Aims: We performed a multicentric prospective study in order to assess the lovastatin and pentoxifylline efficiency administrated in patients with NASH.

Material and Methods: A number of 87 patients with NASH were included in the study, out of which 59 were with NASH and dislipidemia and were treated with lovastatin (10mg/day), and 28 patients were with NASH, but without dislipidemia and were treated with pentoxifylline, 400 mgx3/day.

Results: Regarding the lovastatin - treated group, the following results were obtained: the level of aspartate aminotransferase (AST) decreased after the first and second month of treatment (p=0.0196, p=0.032); the level of alanine aminotransferase (ALT) decreased after the first and second month of treatment (p=0.0335, p=0.021). The gamma-glutamyl transferasise (GGT) level decreased during the two months of treatment (p=0.079, respectively p=0.253). The level of cholesterol decreased after the first and second month of treatment (p=0.00029, respectively p=0.00028). The APRI score of liver fibrosis in the 3 months of treatment, decreased from the average initial value of 0.188 to 0.142, respectively to 0.102 after the second month of treatment (p=0.030).

Regarding the pentoxifylline - treated group, the following results were obtained: the level of AST decreased after the first month and second month of treatment (p=0.018, p=0.16); the level of ALT significantly decreased after the first and second month of treatment (p=0.033, p=0.126). The GGT level decreased after the first and the second month of treatment (p=0.107, respectively p=0.123). The APRI score decreased from the average initial value of 0.1587 to 0.1135 after the first month of treatment and to 0.133 after the second month of treatment (p=0.022).

Conclusions: Both drugs significantly decreased the levels of transaminases in patients with NASH. Lovastatin reduced the cholesterolemia in the dislipidemic patients. The decrease of the APRI score suggests that both drugs may have benefic effects on the liver histology, too. Our research pleads for the individual treatment in patients with NASH, taking into account the present components of the metabolic syndrome.
INTERLEUKIN-6 AND TUMOR NECROSIS FACTOR IN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS

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Introduction: The objective of this study was to assess the possible correlations between the circulating interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) levels and the anthropometric and abdominal fat distribution in patients with non-alcoholic steatohepatitis (NASH) compared with a control group.

Material and Methods: We studied a group of patients with NASH which were hospitalized in two months period in the Medical Departments of the County Clinical Hospital from Sibiu, Romania, to whom we have analysed the BMI (body mass index), abdominal waist line, IL-6 and TNF-alpha levels.

Results: We have studied 36 patients (16 were diagnosed with NASH and 20 patients formed the control group). There was found a correlation between the abdominal waist line and the level of IL6 (r = 0.4834) and between the abdominal waist line and the level of TNF alpha (r = 0.554), at the patients with NASH. There is a correlation between the BMI and the level of IL6 (r = 0.3340) and between the BMI and the TNF alpha level (r = 0.3904), at the patients with NASH. The glicemic level is correlating with IL6 (r = 0.535) and TNF alpha level (r = 0.629) in patients with NASH. There is also, an acceptable association between the triglycerides level and IL6 and TNF alfa in the NASH group (r = 0.297, respectively, r = 0.237). All these correlations were not so relevant in the control group.

Conclusions: The BMI and the abdominal waist line are correlating with the IL6 and TNF alpha levels in patients with NASH. IL 6 and TNF alpha are well correlated with the glicemia and triglycerides levels, in patients with NASH. These correlations are not so significant in the control group. These findings suggest that the pro-inflammatory cytokines are involved in the pathogenesis of NASH.
ARE THE TYPE OF PROBIOTIC STRAINS AND THEIR AMOUNT EQUALLY EFFECTIVE FOR NAFLD PREVENTION: EXPERIMENTAL COMPARATIVE STUDY?

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Introduction: Today probiotics have been suggested as a treatment for the prevention of chronic liver damage, because they prevent bacterial translocation and epithelial invasion, inhibit bacterial mucosal adherence, production of antimicrobial peptides, that results in decrease of inflammation and stimulation of host immunity. However the question about the comparison of efficacy of different probiotic strains, their combination and form (alive or lyophilized) in management of nonalcoholic fatty liver disease (NAFLD) is still open.

Material and Methods: We included 70 rats divided into 7 groups 10 animals in each. Rats of group I were intact. Newborns rats of groups II-VII were injected with monosodium glutamate (MSG) (4 mg/g). The groups III-V received lyophilized monoprobiotics B.animalis VKL, B.animalis VKB, L.casei IMVB-7280 respectively. The group VI received the mix of these three probiotic strains. The group VII was treated with multiprobiotic “Symbiter” containing concentrated biomass of 14 alive probiotic bacteria (Bifidobacterium, Lactobacillus, Lactococcus, Propionibacterium). Administration was started at 4 weeks after birth and continued intermittently two-week course in 2 weeks intervals. To assess morphological changes in liver we used NAS (NAFLD activity score). Lipid extraction from liver was performed according to Folch.

Results: For steatosis stage there was no significant difference between MSG-obesity group and lyophilized groups III-VI strains. But we found significantly lower degree of steatosis (2.3±0.21 vs 0.7±0.15, p<0.001) for alive probiotic group (VII) as compared to MSG-obesity. For both alive and lyophilized probiotic mixtures reduction of lobular inflammation was observed. These histological data were confirmed by the significant decrease of total lipids and triglycerids content in liver approximately by 22-25% in groups treated with probiotic mixtures (VI, VII) compared to MSG-obesity.

Conclusions: Thus, the obtained data suggest failure of NAFLD prevention with lyophilized monoprobiotic strains. Opposite we established the efficacy of probiotic mixture with the preference of alive probiotic strains. It may be related to more pronounced viability of alive strains, their prevention of bacterial translocation, formation of mutualistic interactions in mixtures and therefore synergistic enhancement of single effect.
CERIUM DIOXIDE NANOPARTICLES PREVENT THE NAFLD DEVELOPMENT IN RATS WITH OBESITY THROUGH THE IMPROVEMENT OF PRO/ANTIOXIDANT STATE

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Introduction: One of the pathogenic mechanisms of the nonalcoholic fatty liver disease (NAFLD) is the accumulation of reactive oxygen species which in turn complicate the disease progress. So, antioxidant therapy is necessary for successful treatment of the liver injury. We have paid attention to novel cerium dioxide nanoparticles (CNPs) which have shown promising antioxidant auto-regenerative ability and low toxicity.

Aims: In current study we aimed to investigate the influence of CNPs on liver lipid peroxidation and antioxidant enzymes activity in rats with experimental NAFLD.

Material and Methods: 30 white male rats were divided into 3 groups: control, MSG- and MSG+ CNPs groups. Newborn rats of control group were injected with saline (control), MSG- and MSG+ CNPs groups were injected with monosodium glutamate (4 mg/g) at 2nd-10th day of life subcutaneously in volume 8 µl/g. Since the age of 1 month, rats of group II had been injected with water in a volume of 2.9 ml/kg, MSG+CNPs groups – with 1 mM solution of CNPs (1 mg/kg). Introduction had been performed intermittently (two-week courses alternated with two-week breaks) for 3 months. To assess morphological changes in liver we used NAS (NAFLD activity score). NAS scale total score ≤3 eliminates NASH and confirmed simple steatosis. In liver tissue the content of lipid peroxidation products and enzymatic activity of superoxide dismutase (SOD) and catalase was studied by standard biochemical methods.

Results: In 4-month rats we found significantly lower total score (1.3±0.26 vs 3.6±0.34, p<0.001), degree of steatosis (1.1±0.18 vs 2.1±0.18, p<0.001) manifestation of lobular inflammation (0.2±0.13 vs 1.2±0.2, p<0.001) and ballooning degeneration (0.0±0.0 vs 0.3±0.15, p=0.034) due to NAS in CNPs group as compared to MSG- group. NASH we confirmed only in 30% of rats with MSG-group (p=0.036). CNPs significantly decreased the lipid peroxidation in liver tissue, namely reduced the conjugated dienes content by 27% (p<0.05), TBA-products – by 43% (p<0.05) and Schiff bases – by 21% (p<0.05). It was also revealed the restoration of SOD activity to the control values and decrease of excessive catalase activity.

Conclusions: Due to antioxidant properties CNPs significantly reduce the incidence of NASH and lead to improvement of the main NAFLD histological features.
IMPAIRED PPARALPHA SIGNALLING INFLUENCES BILE ACID HOMEOSTASIS AND INFLAMMATION IN MICE LACKING ATGL (PNPLA2)

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Introduction: Adipose triglyceride lipase (ATGL/PNPLA2) is the main enzyme in intracellular lipolysis and generates fatty acids (FA) such as linoleic acid as PPARα ligands. PPARα activation represses the rate limiting enzyme in bile acid (BA) synthesis Cyp7a1, indicating a key role of PPARα in biliary homeostasis.

Aims: Therefore, we aimed to determine the effects of hepatic ATGL deficiency (and subsequently impaired FA signalling via PPARα) on BA homeostasis.

Material and Methods: Bile flow was analysed in liver specific ATGL knock out (ATGL LKO) and wild type (WT) littermates. Furthermore these mice were subjected to bile duct ligation (BDL) for 7 days (acute cholestasis model). RT-QPCR was used to assess BA synthesis, inflammation and fibrosis gene expression markers. Serum biochemistry and histology were also assessed.

Results: ATGL LKO mice show increased bile flow (p<0.05) compared to WT mice. In line, mRNA levels of Cyp7a1 were 4fold induced (p<0.05). Inflammatory markers F4/80 and iNOs were increased 2.2fold (p<0.05) and 7fold (p<0.05), in line with impaired PPARα-signalling. Importantly, acute cholestasis in ATGL LKO mice increased F4/80 expression about 10fold vs. WT BDL mice (p<0.05). Additionally, mRNA expression of fibrotic marker TGFβ (p=0.087) and elevated proliferation of cholangiocytes reflected by induced CK19 levels (p=0.057) tended to be increased. Notably, mRNA levels of key hepatobiliary (basolateral and apical) BA transporters and levels of serum liver enzymes, cholesterol, TG and BA levels did not differ between groups.

Conclusions: Loss of PPARα activity due to impaired hepatic FA signalling influences BA homeostasis and predisposes to cholestasis-induced inflammation and fibrosis. These findings suggest that ATGL and/or PPARα represent potential therapeutic targets for cholestatic liver disease.
ROLE OF FXR CONTROLLED CHOP IN MEDIATING BILE ACID EFFECTS IN NAFLD

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Introduction: Farnesoid-X-receptor (FXR) has an important regulatory function in hepatic bile acid (BA) and lipid homeostasis.

Aims: We aimed to explore the role of FXR and BAs in the control of Chop (recently suggested to impact on lipid homeostasis via regulation of C/ebpa) as potential regulators of hepatic lipid metabolism and inflammation.

Material and Methods: Wild type (WT) and FXR knock-out (KO) mice were fed a MCD diet to induce NASH. Hepatic gene-expression was profiled by RT-QPCR for markers of hepatic lipid metabolism and inflammation. Moreover, serum biochemistry, liver histology and hepatic TG content were assessed. HepG2 cells were treated with glucose, with/without FXR and RXR agonists. Gene-expression was analysed by RT-QPCR and chromatin-immuno-precipitation was performed.

Results: MCD feeding resulted in increased serum BA levels and liver enzymes in both WT and FXR KO mice. However, Chop mRNA was up-regulated (4-fold) only in MCD fed WT but not in FXR KO mice. Moreover, mRNA levels of C/ebpa were down-regulated (2fold) in MCD challenged WT, but not FXR KO mice. WT MCD mice display a 2,5fold increased VLDL-receptor mRNA expression, consistent with elevated hepatic TG content. Hepatic inflammation in response to MCD (reflected by F4/80, TNFa mRNA expression) was aggravated by absence of FXR. High BA and low glucose levels increased Chop and subsequently repressed C/ebpa expression in a FXR/RXR dependent fashion in HepG2 cells. Finally, a FXR/RXR binding site was identified in the human promoter of Chop demonstrating a highly conserved regulated pathway.

Conclusions: These findings demonstrate that glucose and BAs control Chop expression via FXR/RXR therefore providing novel insights into pathogenesis and treatment of NAFLD.

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IMPACT OF KUPFFER CELLS ON HIGH FAT INDUCED INSULIN RESISTANCE AND LIVER FETUIN-A EXPRESSION

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Introduction: Hepatokines (liver secreted proteins with possible distant action) are emerging potential players in insulin resistance in type 2 diabetic patients.

Aims: Here, we explore the effect of a high fat diet on the expression of fetuin-A, one of those candidate liver proteins, and its relation with liver macrophage (Kupffer cell) activation.

Material and Methods: Male mice of 5 weeks of age were fed a normal diet (ND) or a high fat diet (HFD) for 3 days, known to initiate steatosis and insulin resistance. A preventive Kupffer cell (KC) depletion was obtained by intravenous injection of clodronate loaded liposomes and compared with PBS liposomes. The mRNA and protein expression of fetuin-A was evaluated by RT-PCR, Western-blot and immunofluorescence (IF) on different insulin-sensitive tissues (liver, adipose tissue and muscle).

Results: Short term HFD induced steatosis, KC activation and insulin resistance together with a significant increased expression of liver fetuin-A mRNA (1.5 fold, p<0.01). However, liver fetuin-A protein expression remained unchanged under short term HFD. This increase in fetuin-A under high fat diet was not evidenced in the peripheral insulin sensitive tissues (skeletal muscle and adipose tissue) whether at the mRNA or at the protein level. Kupffer cell depletion in this setting did not reduce hepatic steatosis but significantly ameliorated insulin sensitivity proved by clamp studies. This amelioration in insulin sensitivity in KC-depleted mice was associated with a significant decrease in fetuin-A mRNA expression (0.7 fold, p<0.01) compared to animals with KC. On immunofluorescence, fetuin-A was mostly expressed in centrilobular hepatocytes. Interestingly, while selectively depleting liver macrophages without affecting adipose tissue macrophage infiltration, intravenous clodronate injection was associated with a significant reduction in epididymal adipose tissue expansion compared to PBS injection (1.1% of body weight versus 1.6% of body weight, p<0.001).

Conclusions: This study demonstrates liver fetuin-A overexpression at the initiation of HFD feeding, concurrent with hepatic steatosis and insulin resistance. Targeting KC in this setting improved insulin sensitivity and was associated with a decreased adiposity and a reduced liver fetuin-A expression suggesting that fetuin-A acts as an hepatokine with pro-adiposity and pro-insulin resistance effects.
RAPID IMPROVEMENT OF HEPATIC STEATOSIS AS ASSESSED BY CONTROLLED ATTENUATION PARAMETER AFTER A TWO WEEK PROTEIN-ENRICHED LOW-CALORIE DIET (HEPAFAST)

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Introduction: Non-alcoholic fatty liver disease (NAFLD) has become one of the most prevalent liver diseases. NAFLD increases the risk of fibrosis and cirrhosis and is the most rapidly growing indication for liver transplantation in patients with hepatocellular carcinoma. Previous intervention studies have been hampered by the need for liver biopsies and the lack of alternative inexpensive methods for the quantitative measurement of liver fat contents.

Aims: Hence the primary aim of this study was to avail of the controlled attenuation parameter as quantitative tool for the assessment of therapeutic effects of a two-week low-calorie diet.

Material and Methods: In this prospective single center study, 59 patients with NAFLD received a 14-day low-calorie liver specific diet (HEPAFAST) containing 1000 kcal per day (41% protein, 29% carbohydrate, 24% fat and 6% fiber). The following parameters were assessed at baseline and after 14 days: hepatic fat contents using controlled attenuation parameter (CAP) during transient elastography (FibroScan); body composition with bioimpedance analysis; and serum liver function tests and lipid profiles using standard clinical-chemical assays.
**Results:** All 59 patients (median age 55 years, range 25-78; 51% women; median BMI 31.7 kg/m$^2$, range 22.4-43.5) successfully completed the study. A significant reduction in hepatic steatosis (14.1%; P<0.0001) was observed after only 2 weeks: The median CAP score decreased from 293 dB/m (range 177-400) at baseline to 263 dB/m (100-353). In parallel, BMI decreased significantly (P<0.0001), and 30.5% could be reclassified into a lower BMI category. Moreover, body and visceral fat contents were significantly (P<0.0001) reduced by 7%. Serum triglyceride, total LDL, and the LDL/HDL index as well as g-GT activities also decreased significantly (all P<0.001). Interestingly, 11 patients (72% women) demonstrated a CAP increase by 3% after the 2-week intervention despite improvements in body composition, thus were classified as hepatic non-responders. In contrast, a subgroup analysis of the responders revealed a decrease of 16% in median CAP scores from 308 to 261 dB/m.

**Conclusions:** This elastography-based non-invasive study shows, for the first time, improvements in hepatic steatosis, as quantified non-invasively by CAP, after a short-term protein-enriched low-calorie diet. The dietary intervention not only reduced body weight but improved both body and liver composition in NAFLD. Compartment- and sex-specific HEPAFAST effects should be investigated further.

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EFFECTS OF PROSTEATOGENIC TM6SF2 AND NCAN/SUGPI VARIANTS ON HEPATIC STEATOSIS AND NON-INVASIVE MARKERS OF LIVER INJURY IN PATIENTS WITH CHRONIC LIVER DISEASES

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Introduction: Previously we demonstrated that the frequent genetic variant PNPLA3 p.I148M is associated with increased hepatic steatosis quantified by controlled attenuation parameter (CAP) and fibrosis measured by liver stiffness (LS) in patients with chronic liver diseases (CLD). Recently single nucleotide variants in the TM6SF2 gene (rs58542926) and the NCAN/SUGPI locus (rs10401969) have been reported as additional genetic determinants of fatty liver (Liu et al. Nat Comm 2014; DiStefano et al. Acta Diabetol 2014).

Aims: Here, we investigate the associations between all of these variants and surrogate markers of liver injury, including CAP and LS, in patients with CLD.

Material and Methods: In total, 174 patients with non-viral and non-cholestatic CLD (50% men, age 18–77 years) were recruited for the study. Hepatic steatosis was phenotyped by CAP during transient elastography (FibroScan). Genotyping was performed using Taqman assays. Statistical analyses included exact tests of Hardy-Weinberg equilibrium (HWE), association tests in contingency tables as well as non-parametric tests for continuous variables.

Results: Overall, the median CAP score was 285 dB/m (100–398), indicating moderate and severe steatosis in the majority of patients, but median LS was 6.1 kPa only (1.6–69.1). CAP values correlated with ALT activities (r=0.236, P<0.01) and LS (r=0.174, P<0.05). We observed the following allele frequencies, all of which were in HWE (P>0.05): TM6SF2 [EE] 139 (79.9%), [EK] 33 (19.0%), [KK] 2 (1.1%); NCAN/SUGPI [CC] 137 (78.7%), [CT] 34 (19.5%), [TT] 3 (1.7%). Carriers of the TM6SF2 and NCAN/SUGPI risk alleles presented with significantly (all P<0.05) increased serum ALT (57 U/l (26–168) vs. 44 U/l (9–2106) and 58 U/l (26–168) vs. 44 U/l (9–2106), respectively). In contrast to the common PNPLA3 mutation p.I148M, neither TM6SF2 nor NCAN/SUGPI genotypes were significantly associated with higher CAP or LS, or with an increased risk of presenting CAP ≥ 238 dB/m, which is characteristic for liver steatosis (all P>0.05).

Conclusions: Our study indicates that the TM6SF2 and NCAN/SUGPI variants do not display marked effects on hepatic steatosis, but carriers of the risk alleles present with increased liver inflammation. Given the low frequencies of the TM6SF2 risk genotypes in the general population, variant PNPLA3 remains the clinically most relevant prosteato- and profibrogenic genetic factor.
THE ROLE OF PROBIOTICS IN NSAID-ASSOCIATED LIVER INJURY

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Introduction: NSAID use is associated with a wide range of side effects, the most usual being those involving the gastrointestinal (GI) tract and liver.

Aims: The aim of our work was to study the peculiarities of colonic microbiota in Helicobacter pylori-negative patients who used NSAIDs more than 1 month, to determine a correlation between the intensity of dysbiosis and age, severity of gastric lesions; and to optimize the treatment with the help of probiotics.

Material and Methods: We observed 90 patients with osteoarthritis (OA) who were H. pylori-negative and used NSAIDs more than 1 month. The mean age was 64.1±6.1. For all of them gastroscopy with further morphological examination, laboratory examination (CBC, ALT, AST, bilirubin, GGT, AP) were performed. The fecal microflora has been analysed by bacteriological culture methods. Patients with erosive lesions were divided into 2 equal groups. The first group (control) was treated with pantoprazole (20 mg 2 times daily) for 28 days. The second group (main) received combined therapy: pantoprazole (20 mg 2 times daily) for 4 weeks and probiotic “Symbiter acidophilic” concentrated in dose 10 ml per day for 20 days. Over 1 month after the beginning of treatment we repeated all examinations which were done before.

Results: The mean baseline ALT, AST, and GGT activities were significantly elevated in patients who used NSAIDs to the control group. Changes in colonic microbiota were observed in all patients who used NSAIDs for more than 1 month. In 35% of them the level of Lactobacillus was less than $10^6$. The level of Bifidobacterium depends on the patient’s age ($r=-0.45$, p-value=0,004). The correlation between Lactobacillus and age was not observed ($r=-0.20$, p>0,05). Patients treated with probiotics had significantly lower AST and ALT activity at the end of treatment than those who were treated only with PPI. After 20 days of probiotic therapy, patients had significantly increased numbers of both bifidobacteria and lactobacilli compared to the standard therapy where dysbiosis was even enhanced.

Conclusions: The inclusion of multiprobiotic in the general scheme of treatment of NSAIDs-gastropathy eliminates the detected dysbiosis and improves the indicators of ALT, AST, and GGT and what the important - it leads to the total healing of gastric mucous over 4 weeks from the beginning of treatment that allows us to recommend the inclusion of probiotic in the general scheme of the treatment of NSAIDs-gastropathy.
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COMPARATIVE ANALYSIS OF SERUM LIPIDS IN PATIENTS WITH CHRONIC HEPATITIS C AND B, NONALCOHOLIC FATTY LIVER DISEASE, AND HEALTHY CONTROLS

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Introduction: Serum lipids abnormalities are usually seen in patients with chronic hepatitis C (CHC), but the impact of concomitant steatosis, as well as differences with chronic hepatitis B (CHB), and nonalcoholic fatty liver disease (NAFLD) are not well established yet.

Aims: The aim of the study was to assess and compare the serum lipids in patients with CHC genotype 1 with or without concomitant nonalcoholic steatosis (NAFLD overlap) versus patients with CHB (with or without concomitant nonalcoholic steatosis), NAFLD, and healthy controls (HC).

Material and Methods: A total of 1344 subjects were included in this study: 366 CHC genotype 1 patients with (n=227) or without (n=139) steatosis, 334 CHB patients with (n=160) or without (n=174) steatosis, 403 NAFLD patients, and 241 HC without liver or other diseases, and metabolic syndrome (MS), matched for age and gender. Diagnosis of liver disease was based on standard criteria and confirmed by histology in all cases with viral hepatitis and 141 patients with NAFLD. Serum lipids, BMI, components of MS, and insulin levels were evaluated.

Results: In all patients with steatosis (NAFLD, CHC and CHB) the mean levels of total/LDL-cholesterol and triglycerides were higher than cases without steatosis (HC, CHC and CHB), and those of HDL-cholesterol were lower (p<0.05-0.001). More severe degree of lipids abnormalities was observed in NAFLD vs CHC and CHB with steatosis for total/LDL-cholesterol (p<0.001), as well as in NAFLD and CHB vs CHC with steatosis for triglycerides (p<0.001). Increased total/LDL cholesterol (p<0.001), and decreased HDL-cholesterol (p=0.04) in patients with CHC with steatosis (19%, 14% and 28% respectively) were significantly lower than NAFLD, as well as increased triglycerides (22%) versus NAFLD (p<0.001), and CHB with steatosis (p=0.01). There was no difference between CHC patients without steatosis and HC. The higher frequency and degree of other metabolic abnormalities were also observed in NAFLD and NAFLD overlap on CHC and CHB vs cases without steatosis (p<0.05-0.001).

Conclusions: Lipids and other metabolic abnormalities in patients with CHC, as well as CHB depend on liver steatosis. NAFLD, NAFLD overlap on CHC and CHB are associated with atherogenic type dyslipidaemia, but in CHC total and LDL-cholesterol are the lowest.
DIFFERENCES IN THE INTESTINAL MICROBIOTA IN PATIENTS WITH CHRONIC HEPATITIS C

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Introduction: The importance of the intestinal microbiota in the onset and clinical course of many diseases, including liver diseases like AFLD or NAFLD and liver cirrhosis, is becoming obvious. However, the role of the intestinal microbiota in chronic HCV infections remains unclear.

Material and Methods: We performed a cross sectional study comprising 51 patients infected by HCV (n=18 w/o cirrhosis (NO-CIR); n=28 with cirrhosis (CIR); n=5 after solid organ transplantation (TX)) and 26 healthy controls (HC). Stool samples were prospectively collected, mixed with RNAlater and stored at -20°C. DNA was extracted using TRIzol reagent. The V1-2 region of the 16S rRNA gene was amplified followed by sequencing on the Illumina MiSeq platform. Sequences were quality filtered, trimmed collapsed and clustered and the annotation was performed using the RDP classifier. Statistical analysis was performed using SPSS 22 and Primer 6.

Results: A total of 4,328,784 reads were obtained out of which 3,060,948 (71%) reads passed the quality control giving a median of 35,132±16,611 per sample. The mean Shannon diversity index (H’) was significantly lower in individuals infected with HCV compared to HC (4.22±0.38 vs. 3.97±0.51; p=0.025). In addition, H’ was significantly decreasing in HC, NO-CIR and CIR (4.22±0.38 vs. 4.10±0.51 vs. 3.86±0.53; p=0.037). H’ showed a negative correlation with disease status (HC vs. NO-CIR vs. CIR) and the Child-Pugh score (CPS) (r=-0.322; p=0.006 and r=-0.478; p<0.001). Significant different community structures could be observed for the different stages of disease (HC vs. CO-CIR vs. CIR vs. TX).
Bacilli and Gammaproteobacteria become more abundant during the course of HCV infection and decreased after organ transplantation (p<0.001 and p=0.001; Fig). Concomitantly, Alphaproteobacteria declined during the course of HCV infection and increased after TX (p=0.008). Bacilli and Gammaproteobacteria showed a negative correlation with H’ (r=-0.490; p<0.001 and r=-0.412; p<0.001). In contrast, the frequency of Bacilli showed a positive correlation with CPS (r=0.505; p<0.001).

**Conclusions:** Our cross sectional study reveals significant differences between the microbial communities in patients during different stages of chronic HCV infection. The diversity is decreasing and the frequency of Bacilli and Gammaproteobacteria is increasing. These factors could be used to identify patients at risk of disease progression. However, these results need to be confirmed in long term studies.
TNF-ALPHA SERUM LEVEL, INTRAHEPATIC T-HELPER (TH)17 CYTOKINE AND CD163-POSITIVE MACROPHAGES IN DEVELOPMENT OF LIVER DAMAGE IN PATIENTS WITH NAFLD

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of elevated chronic liver disease in the Europe. Many molecular and cell markers play a major role in development of liver damage and progression of NAFLD.

Aims: The aim of the present study was to evaluate the tissue distribution of IL-17 and CD163 positive cells, to define the level of TNF-alpha and to investigate the relation of these cells and markers with liver damage and progression in patients with NAFLD.

Material and Methods: The immunohistochemical expression of IL-17 and CD163 was evaluated in thirty two patients with NAFLD and 20 healthy controls. Also, we compare the data with our previous investigation of serum concentrations of TNF-alpha by enzyme-linked immunosorbent assay.

Results: We found that the histology of the patient’s livers showed different signs of mild portal inflammation, levels of fibrosis and micronodular steatosis. The density of CD163- and IL-17-positive cells was higher in patient group compare with control group ($\chi^2=10.1$, $p=0.04$). Second, we found that the specimens with high density of CD163-positive cells in portal tracts correlated with the presence of fibrosis in the same places ($\chi^2=6.64$, $p=0.032$). Intrahepatic lymphocytes positive for IL-17 in portal tracts were more in patients with progressive liver damage compare with less marked patients and controls ($\chi^2=12.4$, $p=0.001$). Again, the presence of NAFLD was associated with higher levels of TNF-alpha (OR = 4.26; 95% CI: 1.16 - 15.54; $p = 0.028$).

Conclusions: The infiltration with CD163-positive macrophages and high density of IL-17-positive T-lymphocytes support that these types of cells together with TNF-alpha plays paramount role in liver damage.
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AN OPEN LABEL RANDOMIZED CONTROL STUDY TO COMPARE THE EFFICACY OF VITAMIN E VERSUS URSODEOXYCHOLIC ACID IN NON DIABETIC INDIAN NAFLD PATIENTS

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Introduction: Several studies have investigated UDCA (conventional and high doses) to improve aminotransferases and steatosis in patients with NAFLD and liver histology in patients with NASH. AASLD recommends use of Vitamin E administered at daily dose of 800 IU/day in non-diabetic adults with biopsy-proven NASH and is considered as a first-line pharmacotherapy for this patient population.

Aims: The study was carried out to compare the efficacy of Vitamin E versus ursodeoxycholic acid (UDCA) in non-diabetic NAFLD patients.

Material and Methods: We randomized 250 non cirrhotic and non-diabetic NAFLD patients diagnosed on ultrasound, with raised aminotransferase (ALT) (> 40 IU/L), to receive Vitamin E 400 mg twice a day (Group 1) or UDCA 300 mg twice a day (Group 2) for 52 weeks. Life style modification to achieve at least 5% weight reduction and subsequent weight control and regular exercise was advised to both groups. The primary study endpoint was normalization in ALT levels from baseline. Secondary end points were the proportion of patients with reduction in ALT, relative reduction in the NAFLD Fibrosis score (NFS), symptomatic improvement, and tolerability.

Results: 150 patients received UDCA as compared to 100 patients receiving Vitamin E. The treatment groups were comparable at entry with regard to age (44.1 versus 42.4 years), gender (67% versus 63% female), risk factors for NASH, hypochondriac pain, serum liver biochemistries and NAFLD Fibrosis score. The primary end point was achieved in 21(14%) and 19(19%) of patients in Group 1 and group 2 respectively (p=0.2). The proportion of patients with reduction in ALT (56% versus 63%, p=0.2), symptomatic improvement (78% versus 67%, p=0.058), reduction in the NFS (44% versus 47%, p=0.69) and tolerability (98% versus 95%, p=0.2) were similar between Group 1 and Group 2 respectively.

Conclusions: UDCA is an effective and safe alternative to Vitamin E in non diabetic–non cirrhotic Indian NAFLD patients.
PLATELET COUNT FOR PREDICTING LIVER FIBROSIS IN NAFLD/NASH WITH TYPE 2 DIABETES

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Introduction: The severity of liver fibrosis is known to be one of the good indicators for determining the prognosis, for surveillance, and for optimal treatment of NAFLD. However it is virtually difficult to enforce liver biopsy in NAFLD patients with type 2 DM who are followed up by DM department.

Aims: To investigate the clinical usefulness of measuring the platelet count for predicting the severity of liver fibrosis in NAFLD patients with type 2 DM.

Material and Methods: A total of 1,800 patients with liver-biopsy-confirmed NAFLD seen between 2002 and 2012 were enrolled from ten hepatology centers in Japan. Of these, NAFLD with type 2 Diabetes were exist in 643 (35.7%) patients (Stage 0: 66, Stage 1: 177, Stage 2: 203, Stage 3: 164, Stage 4: 33). Laboratory evaluations in all patients were performed.

Results: A linear decrease of the platelet count with increasing histological severity of hepatic fibrosis was revealed. By multilogistic regression analysis, the platelet count were selected as independent variables associated with severe fibrosis (Stage 3-4) in NAFLD patients with type 2 diabetes. The AUROC curve estimating the diagnostic performance of the platelet count for hepatic fibrosis stages 3 was 0.724 (optimal cut-off value, 20.1 x 10^4/ml; sensitivity, 64.0%; specificity, 68.8%), and that for stage 4 was 0.864 (optimal cut-off value, 16.5 x 10^4/ml; sensitivity, 72.7%; specificity, 82.0%).

Conclusions: The platelet count may be an ideal biomarker of the severity of fibrosis in NAFLD patients with type 2 DM, because it is simple, easy to measure and handle, cost-effective, and accurate for predicting the severity of fibrosis. Furthermore, by using cut-off-value of platelet counts validated in our multiple large trials, mass screening of NAFLD patients with type 2 DM may be facilitated, especially followed by DM department.
DIFFERENTIAL IMMUNOMETABOLIC PHENOTYPE IN TH1 AND TH2 DOMINANT MOUSE STRAINS IN RESPONSE TO HIGH-FAT FEEDING

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Introduction: Evidence is accumulating that immune reactivity plays an important role in metabolic diseases.

Aims: Therefore, we have investigated the strain-dependent differences of adipose tissue and liver immunophenotype in high-fat diet induced obesity, liver steatosis and fibrosis, and glucose metabolism in the two, C57Bl/6 and BALB/c, prototypic Th1 and Th2 mouse strains.

Material and Methods: Male C57BL/6 and BALB/c, 8-week old mice received HFD (60% kcal fat) or standard chow diet (10% kcal fat) for 24 weeks. We performed histological and immunophenotypic analyses as well as gene expression of profibrogenic and lipid metabolism-related molecules in liver and adipose tissue.

Results: After 24 weeks of dieting BALB/c mice had higher weight gain on standard diet, while C57Bl/6 mice exhibited higher weight gain on HFD. The amount of visceral fat and fasting blood glucose levels were higher in C57Bl/6 mice on both diets. In contrast to BALB/c mice, HFD in C57Bl/6 mice led to a significant increase of the amount of VAT and number of VAT associated CD3+CXCR3+ Th1 cells, dendritic cells (DCs) and F4/80+ macrophages. In livers, higher number of CD3+ and CD8+ T lymphocytes, myeloid DCs, proinflammatory macrophages (F4/80+CD11b+CD11c+ and F4/80+IL-1β+) and CD11b+Ly6C+ monocytes and higher levels of IL-6, TNF-α and IFN-γ were detected in HFD-fed C57Bl/6 mice than in BALB/c mice. C57Bl/6 mice which had scarce liver steatosis, while BALB/c mice showed prominent high-fat diet induced liver steatosis, associated with increased expression of genes related to lipid metabolism and higher serum levels of cholesterol and triglycerides with lower glycogen deposition in the liver. In contrast, BALB/c mice developed scarce liver collagen deposition while C57Bl/6 mice had prominent liver fibrosis. The expression of genes related to fibrosis such as procollagen, IL-13 and TGF-β and the levels of profibrogenic cytokines IL-13 and TGF-β in sera and liver homogenates were significantly higher in C57Bl/6 mice with the most prominent increase in liver IL-33 levels.

Conclusions: Th1 type response appears to favour inflammation and fibrosis in the liver while mice with dominant Th2 response are prone to steatosis.
METHYLATION AND EXPRESSION OF PNPLA3, SAMM50, AND PARVB GENES INFLUENCE ON NONALCOHOLIC FATTY LIVER DISEASE

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Introduction: Genetic Variations in the genomic region containing patatin-like phospholipase domain containing 3 (PNPLA3), SAMM50 sorting and assembly machinery component (SAMM50), and parvin, β (PARVB) are important for the development and progression of nonalcoholic fatty liver disease (NAFLD). In many common disease, epigenetics is now recognized as an important factor, in addition to a genetic factor.

Aims: We analysed the association the histological severity of NAFLD with the methylation levels of CpG99, CpG71, CpG26, and CpG101, and with the mRNA levels of PNPLA3, SAMM50 and PARVB.

Material and Methods: We performed the targeted-bisulfite sequencing and measuring the DNA methylation in 4 CpG islands (CpG99, CpG71, CpG26, and CpG101) which exist in the upstream of PNPLA3, SAMM50, PARVB variant 1, and PARVB variant 2, respectively. We compared the DNA methylation in the liver and blood of 22 mild (fibrosis stage 0 and 1) and 11 advanced (fibrosis stage 2 and 3) NAFLD patients.

Results: We found that CpG26, which exists in the upstream of PARVB variant 1, was remarkably hypomethylated in the liver of advanced NAFLD. Hypomethylation was also associated with the increased hepatocyte ballooning score. CpG99 in the 5’-region of PNPLA3 was hypermethylated in the advanced NAFLD group. CpG99 hypermethylation was also associated with increased hepatocyte ballooning and lobular inflammation scores. These differential methylations were not observed in the blood DNA. The mRNA levels of PNPLA3 were lower in the liver of the advanced NAFLD group and inversely correlated with liver DNA methylation levels in CpG99. The mRNA levels of SAMM50, and PARVB variant 2 were not different between mild and advance NAFLD. PARVB variant 1 was expressed in the liver: however, the mRNA levels could not be measured.

Conclusions: Hypermethylation in CpG99 would decrease the expression of PNPLA3. Hypomethylation in CpG26 and hypermethylation in CpG99 would be related NAFLD severity, through changing the mRNA expression levels.
A PURELY DIET-BASED MOUSE MODEL OF NASH THAT PRODUCES SEVERE INFLAMMATION AND FIBROSIS

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Introduction: The optimal models that reflect all features of human NASH are urgently needed.

Aims: We developed a NASH model using a diet which rich in transfats, carbohydrates, and cholesterol (nutritional stress diet, NSD) that mimics major features of human fibrotic NASH.

Material and Methods: Female 6 wk old C57BL/6 mice (n=10 per group) were fed a control diet (13% kcal fat; 65% kcal carbohydrates), or a high saturated fat diet enhanced in fructose and cholesterol (nutritional stress diet, NSD: 59kJ% kcal lipid, hydrogenated coconut oil; 26kJ% kcal carbohydrates, fructose and glucose; 15kJ% kcal protein, casein; 2% cholesterol), plus fructose-sucrose in drinking water (12.6%: 55:45) for 30 weeks. At sacrifice, liver function, lipid accumulation, IR, inflammation, fibrosis, oxidative stress, and mRNA levels related to fat metabolism, inflammation, fibrogenesis, fibrolysis, and macrophage polarization were analysed by IHC and qRT-PCR.

Results: NSD fed mice displayed a significant increase in body (mean 42g vs 25g) and liver weight (mean 2.31 vs 1.14g), insulin resistance (HOMA-index 1.4-fold), steatosis (grade 3 vs 1), hepatocyte ballooning, and inflammation (NAS score 4), fibrosis (Ishak score 3), and hepatic cholesterol content (2.2-fold). Serum parameters of liver injury, ALT, AST and LDH were elevated 12-, 2.1-, and 3.1-fold, respectively, and hepatic collagen (hydroxyproline) was increased 5.7-fold. This was paralleled by a significantly increased expression of fatty acid metabolic enzymes, and transcripts related to inflammation, macrophage M1/M2 polarization, fibrogenesis, and fibrolysis (PPARG, LPL, TNFA, CCL5, MCP1, COL1A1, ACTA2, TGFB1, ITGB6, PDGFRB, PAI1, SPP1, TIMP1, MMP2, MMP8, MMP13). The visceral (gonadal) fat pad of NSD fed mice was significantly increased in size, with 3- to 5-fold upregulated TNFα and IFNγ transcript levels compared to controls. As assessed by IHC, the oxidative stress markers 4-hydroxynonenal and 8-hydroxyguanosine were highly overexpressed in livers of mice fed the NSD.

Conclusions: We describe an optimized diet-induced mouse model that produces all features of human NASH, with robust inflammation and fibrosis. This model should permit an optimized preclinical testing of novel agents that address the major pathologies of human NASH.
CONTROLLING HEPATOCYTE LIPID DROPLET PROTEINS THROUGH REGULATION OF SREBP-1C AND CAVEOLIN-2 BY MIR-29A IN HCV INFECTED CELL MODELS

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Introduction: Hepatitis C Virus (HCV) is a major causative agent of Non-alcoholic fatty liver disease (NAFLD) which is characterized by excessive accumulation of lipid droplets (LDs) in the cytoplasm of the cells which the virus utilizes to promote its life cycle. Sterol Regulatory Element Binding Protein-1c (SREBP-1c) is a lipogenic transcription factor induced in response to HCV infection promoting LD formation. Moreover, Caveolin-2 (CAV-2) is a lipid raft associated intracellular membrane protein that is suggested to play a role in HCV replication.

Aims: The aim of this study is to examine the regulation of SREBP-1c and CAV-2 by miRNAs in HCV infected models in an attempt to reduce HCV induced NAFLD.

Material and Methods: Bioinformatic analysis was performed and revealed that miR-29a potentially targets the 3’UTR of SREBP-1c and CAV-2. Expression profile of miR-29a, SREBP-1C and CAV-2 was examined in JFH-1 infected, oleic acid (OA) treated (Group-A) versus untreated (Group-B) Huh-7 cells. Furthermore, Group-A and Group-B were manipulated using mimics and inhibitors of miR-29a to observe their impact on the expression of SREBP-1C and CAV-2 using quantitative real time PCR (qRT-PCR). In Group-A OA was added 24 hours following transfection to induce the formation of LDs.

Results: miR-29a was significantly up regulated in Group A versus Group B (p=0.0215), however, there was no difference in the expression level of neither SREBP-1c nor CAV-2. Mimicking of miR-29a in (Group A) resulted in up regulation of SREBP-1c (p=0.0447) with no significant change in CAV-2, however, mimicking of miR-29a in Group-B resulted in down regulation of CAV-2 (p=0.0346) with no significant change in SREBP-1c. Since SREBP-1c is up regulated after mimicking with miR-29a this may give an indication that the 5’UTR of SREBP-1c is targeted by miR-29a, which was shown to be a potential target site by BIBISERV software.

Conclusions: SREBP-1c expression is induced by miR-29a which is associated only with increased LD formation and may be through targeting the 5’UTR, while CAV-2 expression is suppressed by miR-29a through targeting the 3’UTR. Therefore, miR-29a is a potential controller of lipid droplet metabolism through targeting important LD proteins.
SHORT-TERM VITAMIN D SUPPLEMENTATION IMPROVES HEPATIC STEATOSIS AS QUANTIFIED BY CONTROLLED ATTENUATION PARAMETER (CAP)

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Western countries. A recent meta-analysis confirmed decreased serum 25-hydroxyvitamin D levels in patients with NAFLD. Vitamin D deficiency is suggested to increase the activity of toll-like receptors, which affect the gut microbiome and are implicated in the pathogenesis of NAFLD.

Aims: To investigate whether vitamin D therapy ameliorates hepatic steatosis in patients referred to the outpatient liver clinic of a tertiary centre.

Material and Methods: We prospectively recruited patients with NAFLD and vitamin D deficiency (defined by serum concentrations <20 ng/ml). Hepatic steatosis was assessed using the controlled attenuation parameter (CAP), which quantifies the degree of ultrasound attenuation by liver fat during vibration-controlled transient elastography (FibroScan). Patients were included if they had significant liver fat accumulation (defined by a CAP score ≥ 280 dB/m). Serum 25-hydroxyvitamin D were measured by chemiluminescent immunoassays. Body composition was determined with bioelectrical impedance analysis. Currently, 32 patients entered the intervention arm of the study and received 20,000 IU vitamin D daily for 7 days, thereon weekly for 6 months.

Results: The cohort comprised 48.7% women (mean age 54±12 years; mean BMI 29.5±3.1 kg/m²). Moderate vitamin D deficiency was present in 59.5% and severe vitamin D deficiency (<10 ng/ml) in 40.5% of patients. Fatty liver quantification showed a mean CAP of 328.5±30.9 dB/m. The CAP score was significantly lower in patients with severe vitamin D deficiency (342.8±31.8 vs. 317.3±27.0 dB/m, P=0.013). A rapid increase in vitamin D levels was noted after 4 weeks of vitamin D supplementation (33.2 vs. 10.9 ng/ml, P<0.0001), with 73% of patients displaying normal levels (>30 ng/ml). CAP scores significantly decreased by 5% at the 4-week interval (312.8 vs. 328.5 dB/m, P=0.047). During this time-period liver function tests, BMI and body fat levels remained unchanged. Patients will be monitored again at 3 and 6 months.

Conclusions: Vitamin D levels correlate with the degree of hepatic steatosis, which significantly improves after only 4 weeks of vitamin D replacement therapy. We conclude that hepatic steatosis as assessed by CAP is a dynamic process, which appears to be modulated by short-term therapeutic interventions such as vitamin D substitution. The molecular mechanisms underlying hepatocellular lipid remodelling by vitamin D remain to be identified.
Non-alcoholic fatty liver disease is associated with alterations in lipidomic profiles

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Introduction: The pathogenesis of NASH is driven in part by lipotoxicity. Evaluation of the lipid metabolites profiles in individuals with NASH may aid in further understanding of the pathogenesis of NASH.

Aims: We assessed differences in the serum lipidomic profiles between BMI-matched women with normal liver histology and histologically-confirmed NASH.

Material and Methods: Eighteen women aged 18-65 years underwent liver biopsy and blood draw at the time of weight loss surgery. Women with diabetes, alcohol use or other chronic liver disease were excluded. Patients with NASH were BMI-matched to those with normal liver histology. A second cohort of 18 women served as a validation cohort. Lipid species were extracted from plasma and analysed by HPLC-RP column coupled 4000-QTRAP mass spectrometry.

Normal histology was defined as the absence of steatosis, inflammation, ballooning or fibrosis. NASH was defined as score of >=1 in steatosis, lobular inflammation and ballooning.

Lipidomics data was pre-processed using Matlab by imputing missing values from group means or from floor value corresponding to the limit of detection. Data was log transformed to ensure normal distribution. Quantile normalization was performed to make the distribution of scores for samples identical, followed by T-test for differences between groups.

Results: NASH was associated with a >1-fold decrease in phosphatidylinositol (PI) C34:0; sphingomyelins (SM) C20:1, C21:0, C21:1, C23:0, C23:1; phosphatidylethanolamines (PE) C32:1, C34:0, C34:2, C36:0; lysophosphatidylethanolamines (LPE) C14:0, C18:0, C22:6 and phosphatidycholines (PC) C30:2 C32:2, all of which were statistically significant. (Figure 1) These folds changes remained significant and greater than 1-fold less in the validation cohort.
NASH was also associated with significant increases (>1x-fold) in 9 species of triacylglycerols (TAG) and 4 types of diacylglycerols (DAG) (Figure 1) which remained significant with >1-fold change in the validation cohort.

**Conclusions:** Several species of lipids including SM, PE and LPEs were significantly lower in women with NASH, suggesting possible hepatic sequestration. Further, several species of DAGs and TAGs were significantly increased and may contribute to the increased CVD risk seen in NAFLD. Obese women with NASH have unique lipid metabolite profiles from BMI-matched women with normal liver histology. Further evaluation is needed to determine whether lipidomic profiles differ between individuals with steatosis and NASH.
AKKERMANSIA MUCINIPHILA PROTECTS FROM EXPERIMENTAL ALCOHOLIC LIVER DISEASE

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Introduction: Alcoholic liver disease (ALD) represents the hepatic manifestation of alcohol overconsumption and is a major reason for liver disease in the western world. ALD includes a clinical spectrum of alcoholic fatty liver, alcoholic steatohepatitis (ASH), cirrhosis and hepatocellular carcinoma. ASH is a disease state of severe inflammation and shows a mortality up to 50%. An important pathomechanism in the development of ASH is the translocation of pathogen-associated molecular patterns (PAMPs, i.e. LPS, microbial DNA) from the gut into the liver. Once in the liver, PAMPs activate numerous pathways that promote hepatic steatosis, inflammation and fibrosis. An intact gut barrier is maintained by a continuous interaction between the gut microbiota and the mucosal immune system. Members of the gut microbiota such as Akkermansia muciniphila affect mucosal thickness what might decrease portal and systemic LPS levels.

Aims: The aim of our study was to investigate the impact of Akkermansia muciniphila on the development of ASH in mice.

Material and Methods: 6-8 week old female wildtype mice were fed daily a Lieber-DeCarli diet increasing an ethanol concentration stepwise from 0 vol% to 5 vol% or fed an isocaloric diet without alcohol. Additionally, the mice were treated three times per week with 1,5x10^8 CFU Akkermansia muciniphila by oral gavage or an equivalent volume of PBS. Accordingly, control groups received an isocaloric diet without alcohol and Akkermansia muciniphila or vector control. On day 15, all mice were sacrificed and samples of serum, liver, colon and microbiota were analysed.

Results: Treatment with Akkermansia muciniphila significantly reduced the mean GPT [U/l] in the alcohol group (p=0.0096). Mice treated with alcohol and Akkermansia muciniphila also showed a trend towards a reduction of proinflammatory cytokine expression in the liver including TNF-alpha, IL-1beta, MIP-2, IL-6 and Itga-4. The improved pathogenic phenotype in Akkermansia muciniphila treated mice could be explained by an altered gut-barrier; mRNA levels of colon samples showed a significant increase of barrier-forming Cldn-3 (p=0.0047) and Muc1 (p=0.0488).

Conclusions: This study reveals new insights into a potential role for Akkermansia muciniphila contributing to experimental alcoholic liver disease. Our results suggest a rationale for the use of an Akkermansia muciniphila-based probiotic in humans with alcoholic liver disease and especially ASH.
CERIUM DIOXIDE NANOPARTICLES ATTENUATE THE INFLAMMATION IN RATS WITH NAFLD

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Introduction: The inflammatory processes are implicated in pathogenesis of the liver injury and nonalcoholic fatty liver disease (NAFLD) associated with obesity. Proinflammatory cytokines lead to the liver tissue destruction and fibrosis that worsens the obesity consequences and complicates the liver treatment. So, the search of non-toxic drugs to prevent obesity sequelae namely inflammation is of the current interest.

Aims: The aim of the study is to estimate the anti-inflammatory properties of cerium dioxide nanoparticles (nCeO₂) on the rat model of NAFLD associated with monosodium glutamate (MSG)-induced obesity.

Material and Methods: The study was carried out on 30 male rats that were divided into control, MSG- and MSG+nCeO₂ groups. Newborn rats of control group were injected with saline (control), MSG- and MSG+nCeO₂ groups were injected with MSG (4 mg/g) at 2-10 days of life subcutaneously in volume 8 µl/g. Since the age of 1 month, rats of group II had been injected with water in a volume of 2.9 ml/kg, MSG+nCeO₂ groups – with 1 mM solution of nCeO₂ (1 mg/kg). Administration of nCeO₂ had lasted for 3 months (3 two-week courses) until 4-month age. After that rats were sacrificed and visceral adipose tissue (VAT) and blood were harvested. The content of proinflammatory cytokines (interleukin (IL)-1β, IL-12Bp40, interferon-γ (INF-γ)) and anti-inflammatory cytokines (IL-4, IL-10, tumor growth factor-β (TGF-β)) were measured by ELISA.

Results: In 4-month rats injected with MSG we determined the development of visceral obesity that was confirmed by the increase of the VAT mass (control vs. MSG-group 4.7±0.6 g vs. 19.0±2.0 g, p<0.05). CNPs significantly decreased the VAT mass to 8.3±1.4 g (p<0.05). We observed a significant increase of IL-1β and IL-12Bp40 content in blood serum of obese rats that confirms the inflammation in MSG-injected animals. nCeO₂ attenuated inflammation that was evident by decrease IL-1β by 12.5% (p<0.05) and IL-12 by 23.7% (p<0.05) compared with MSG-group. Opposite the level of INF-γ did not change in all three groups. The content of anti-inflammatory IL-4 and TGF-β in obese rats was decreased and the level of IL-10 was elevated because of compensatory reaction of anti-inflammatory system. Studied compound restored the anti-inflammatory cytokines content to the level of control values.

Conclusions: Thus, nCeO₂ attenuates the inflammatory processes in rat blood that can prevent obesity complications and liver injury. Antioxidant action may be one of the mechanisms of such effect of nCeO₂.
ENDOTHELIAL DYSFUNCTION, SYSTOLIC BLOOD PRESSURE, SERUM LEVELS OF AMINOTRANSFERASES IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE IN COMBINATION WITH CORONARY HEART DISEASE

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease. Coronary artery disease followed by liver-related mortality are the most common causes of death in patients with NAFLD. Endothelial dysfunction and cardiovascular risk describe in patient with NAFLD.

Aims: To compare the relationship of endothelial dysfunction, systolic blood pressure, serum levels of aminotransferases in patients with nonalcoholic fatty liver disease in combination with coronary heart disease.

Material and Methods: We studied 19 patients with NAFLD in combination with coronary heart disease mean age 55.8±6.05 (group A) and 9 patients with coronary heart disease without a NAFLD mean age 58.8±5.1 (group B). Patients with diabetes were excluded. The reactive hyperemia test for assessment of endothelial dysfunction was consecutively performed in all patients. Brachial artery enlargement by less than 10% was considered as a sign of endothelial dysfunction. Studied biochemical parameters: aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT).

Results: According to the reactive hyperemia test data, endothelial dysfunction was found in 18 patients (94%) in a group A and in 4 patients (44%) in a group B, vasospastic response observed in 2 patients (10%) in a group A. Reactive hyperemia index (RHI) was lower (4.94±4.3%) in the group A than in group B (9.3±3.4%) (p=0.04). The mean level systolic blood pressure (BP) in a group A was higher (151.2±4.3 mm Hg) than in group B (132.3±3.4) (p<0.05). The mean level diastolic BP changes did not significantly differ between the two groups (94.5±8.7 mm Hg and 93.6±7.4 mm Hg, p=0.28). The mean value of AST in a group A was higher (24.7±3.2mmol/l) than in a group B (20.1±3.16mmol/l) (p<0.05); of ALT: 32.3±11.6mmol/l and 21.0±7.6mmol/l (p<0.05); of GGT 60.5±9.06mmol/l and 28.0±4.3mmol/l (p<0.05). There was correlation between the endothelial dysfunction, level of AST (r=0.59; p<0.05) and level of GGT (r=0.51; p<0.05) in a group A however in a group B such connection was not observed.

Conclusions: In patients with NAFLD in combination with coronary heart disease endothelial dysfunction were more expressed than in those who had no NAFLD. There was correlation between the endothelial dysfunction, level of AST and level of GGT in patients with NAFLD in combination with coronary heart disease.
DIRECT GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISM AMELIORATES STEATOHEPATITIS AND FIBROSIS IN MODELS OF NASH AND BILIARY FIBROSIS VIA REGULATION OF LIVER MACROPHAGE INFILTRATION AND ACTIVATION

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Corresponding author’s e-mail: wang@uni-mainz.de

Introduction: Glucagon-like peptide-1 (GLP-1) improves insulin sensitivity via enhanced glucose-dependent insulin secretion, inhibition of glucagon release, and delayed gastric emptying following its release into the circulation from the gut.

Aims: We aimed to explore the utility of the long-acting GLP-1 receptor agonist BYDUREON (BY) to address both inflammation and fibrosis in models of NASH and biliary fibrosis.

Material and Methods: BY was administered twice weekly by subcutaneous injection of 0.4 or 2 mg/kg to Mdr2KO mice, and to C57BL/6 mice fed a methionine and choline deficient (MCD) diet for 4 weeks. Hepatic fibrosis was assessed by morphometric analysis of Sirius red stained collagen and measurement of hydroxyproline content. Hepatic inflammation was measured by semiquantitative immunohistochemistry. Fibrosis and inflammation related transcript levels were quantified by quantitative real-time polymerase chain reaction (qPCR). Ex vivo analysis of hepatic inflammatory cells was performed by FACS.
**Results:** In the MCD and Mdr2-/− model, BY treatment significantly lowered elevated serum liver enzymes and decreased collagen content, fibrosis and inflammation related transcripts and protein levels including αSMA, CD68, CCL3, and TNFα, while it increased the (anti-inflammatory) macrophage markers Arg1 and Ym1. BY treatment also reduced the IHC expression of procollagen type III, CD68, F4/80, Ym1 and caspase 3. With BY treatment the number of liver infiltrating CD45⁺CD11b⁺ (myeloid, innate) immune cells and of F4/80⁺ Kupffer cells was reduced and there was a significant decrease of hepatic CD11b⁺Ly6C⁺high cells, indicating less proinflammatory monocyte/macrophage infiltration as compared to mice that received the MCD diet without BY. Differences were even more pronounced for the ratio of CD11b⁺Ly6C⁺low or CD11b⁺Ly6C⁺high vs CD45⁺ cells.

**Conclusions:** Treatment of MCD diet treated and of Mdr2KO mice with BY reduced parameters of hepatic steatosis, inflammation, fibrosis and apoptosis, without negative effects on weight gain, liver or plasma parameters. These results demonstrate that treatment with BY attenuated liver injury through inhibition of inflammatory and apoptosis pathways that govern NASH and fibrosis and support further clinical evaluation of the utility of GLP-1R agonists for treatment of NASH and liver fibrosis.
CHARACTERIZATION OF AN IN VIVO MODEL OF JUVENILE NON-ALCOHOLIC FATTY LIVER DISEASE

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Introduction: The booming increase worldwide prevalence of pediatric Non-Alcoholic Fatty Liver Disease (NAFLD) is worrisome and draws attention.

Aims: Characterized an in vivo model of juvenile NAFLD.

Material and Methods: Male (M) and female (F) C57BL/6J mice were randomly assigned immediately after weaning to control or high-fat high-carbohydrate diet (HFHCD). Animals had access ad-libitum to food for 16 weeks. Body-weight, glycaemia, insulinemia, triglycerides, total cholesterol, HDL-C, ALT, AST, liver histology were screened every 4 weeks and compared to control animals (CTRL).

Results: Soon after the first week, HFHCD induced in both genders a significant gain of bodyweight. Males, after 4 weeks presented also hyperplasia of epididymal fat-pads and only after week 12th, a significant hepatomegaly. As in Table 1, males showed earlier alteration of glycemia, insulinemia as well as lipid profile and ALT. Interestingly, comparable body/blood alterations were observed in females only at the 16th week. Liver histology showed in both genders a mixed macro-microvesicular steatosis steadily increasing after the 8th week. Inflammatory cells foci were observed in males from the very beginning and sustained over the time with presence of ballooning cells towards the 16th week. On the contrary this inflammatory pattern was absent in females. Surprisingly, both genders developed progressive fibrosis starting from the 8th week and rising steadily over the time.

Conclusions: This juvenile model of diet-induced NAFLD progresses faster than those reported in adults. A clear gender difference was found in the onset of the liver injury. Even though the final outcome (fibrosis) was comparable between genders, males presented early signs of liver injury, while females do not.
**Acknowledgements:** This study was supported by MIUR (Art.13 D.LGS 297/99-Progetto Nutrizione e Salute and FIF inhouse grant.

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<thead>
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<th>Parameters</th>
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<td>Body weight</td>
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^a p<0.05; ^b p<0.01; ^c p<0.001
PROGRESSION FROM NAFLD TO NASH: GENDER DOES REALLY MATTER?

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Introduction: NAFLD is most prevalent among males, but NASH has been reported more prevalent among middle-aged women.

Aims: The aim of this study is to assess the events involved in the progression of the disease in both genders.

Material and Methods: Male and female C57BL/6J mice, immediately after weaning were randomly assigned to either control diet (CTRL) or high-fat high-carbohydrate (HFHCD). Animals had ad-libitum access to these diets for 16 weeks, thereafter blood and liver samples were collected for further analysis (histology, gene expression and products of lipoperoxidation). Data was compared vs animals of each gender fed with CTRL diet.

Results: HFHCD induced weight gain, hyperplasia of adipose tissue, hepatomegaly, alterations in glycaemia, lipid profile and ALT both in males and females; hyperinsulinaemia was present exclusively in males (Table 1). Histological analysis showed mixed macro- micro vesicular steatosis in both genders, in line with the increased expression of DGAT2 (important in the final step of triglycerides synthesis), LDLR, receptor and SREBP-1c, both involved in the lipogenesis. Likewise, both groups developed sinusoidal/periportal fibrosis with increased activation of hepatic stellate cells (\(\alpha\)-SMA). Surprisingly, only males showed inflammatory cells foci with some ballooning and increased expression of TNF-\(\alpha\), whereas females presented only an increased lipid peroxidation (absent in males) with no signs of inflammation.

Conclusions: Altogether these data suggest that even if the onset of fibrosis is similar between genders, the mechanism of liver injury is different. Whereas in males is associated to insulin resistance and inflammation, in females is related with an increase of the oxidative process.
**Acknowledgements:** This study was supported by MIUR (Art.13 D.LGS 297/99-Progetto Nutrizione e Salute and FIF inhouse grant.

<table>
<thead>
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<th>Table 1- Summary of the obtained data</th>
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<th>HFHCD Female</th>
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<td><strong>Means±SD</strong></td>
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<tr>
<td><strong>Body weight</strong></td>
<td>1.43±0.15 c</td>
<td>1.46±0.13 c</td>
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<td><strong>Liver weight</strong></td>
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<tr>
<td><strong>Adipose tissue weight</strong></td>
<td>3.12±0.40 c</td>
<td>3.20±0.34 c</td>
</tr>
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</table>

**Serum Parameters**

| Glucose | 1.65±0.29 c | 1.64±0.19 c |
| Insulin | 7.30±4.30 b | 1.75±0.30   |
| Cholesterol | 1.50±0.17 c | 7.3±4.3 c   |
| HDL     | 1.52±0.14 c | 1.52±0.24 c |
| LDL     | 1.70±0.20 c | 2.40±0.50 c |
| ALT     | 3.15±0.88 b | 1.88±0.65 a |
| AST     | 2.30±0.33 b | 1.75±0.27   |

**Gene expression**

**Lipid metabolism**

| DGAT2   | 1.64±0.31 a | 2.25±0.06 c |
| LDLR    | 2.09±0.51 a | 1.72±0.08 a |
| SREBP-1c | 1.35±0.16 a | 1.65±0.31 a |

**Inflammatory response**

| TNF-α   | 2.16±0.94 a | 1.13±0.25  |

**Fibrosis**

| α-SMA   | 2.03±0.48 a | 2.28±0.37 a |

**Liperoxidation**

| MDA     | 0.87±0.29   | 3.82±0.40 a |

\[ a \ p<0.05, \ b \ p<0.01, \ c \ p<0.001 \]
THE IMPORTANCE OF THE INTERPLAY BETWEEN HEPATOCYTES AND HEPATIC STELLATE CELLS DURING FIBROGENESIS IN A NASH IN VITRO MODEL

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Introduction: Activation of hepatic stellate cells (HSC) and dysregulation of several mediators such as matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) play a determinant role in the fibrogenesis during the progression of NAFLD to NASH.

Aims: This study was aimed to establish the interplay between hepatocytes and HSC in an in vitro cell model of NASH.

Material and Methods: The effect of free fatty acids (FFA) (Oleic:Palmitic, 2:1) was analysed at short (24h) and long (96h) exposure times in different experimental set-ups: 1) Monoculture of each cell type; 2) Transwell system (soluble mediators effects) and 3) simultaneous co-culture (SCC) by seeding both cell types together (cell-to-cell interaction). In each system was assessed the amount of steatosis; expression of HSC activation marker (α-SMA), ECM turnover regulators (MMP-2 and TIMP-2) as well as collagen biosynthesis in comparison to untreated cells (ctrl).

Results: The amount of steatosis was comparable among all the experimental set-ups. However, HSC activation in terms of α-SMA gene (2.20±0.25-folds; p<0.01) and protein (1.70±0.20-folds; p<0.01) expression was only increased in the SCC and was maximal after 24h of FFA exposure. Similarly, the close contact of the two cell types induced an up-regulation of TIMP2 protein (1.42±0.27-folds; p<0.05) which was inversely correlated both with MMP-2 protein (0.58±0.10-folds; p<0.01) and activity (0.70±0.13-folds; p<0.05). This dysregulation was accompanied by an increase of collagen biosynthesis at longer FFA exposure times (1.5 ± 0.10-folds, p<0.01). Any of these effects was directly induced by FFA (monoculture) nor by the soluble mediators (transwell).

Conclusions: Our data suggest that hepatocytes-to-HSC proximity/interaction is essential for fibrosis initiation in NASH.

Acknowledgements: Authors are thankful to Dr. Friedman SL, for kindly providing LX-2 cells, Dr. Bestagno, M. (ICGEB) for the support in flow cytometry determinations and to Dr. Bembi’s group for providing the human skin fibroblasts.
A FREQUENT PNPLA3 VARIANT P.I148M IMPROVES RESPONSE TO BARIATRIC SURGERY IN OBESE PATIENTS WITH FATTY LIVER DISEASE

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Introduction: Obesity is the major trigger of fatty liver disease (FLD). This disorder is further favored by the PNPLA3 (adiponutrin) p.I148M genotype, which may also predispose to liver cirrhosis and HCC (Krawczyk et al. Semin Liver Dis 2013). Currently, bariatric surgery is becoming a more frequent weight loss therapy, however its effects on liver status with respect to the PNPLA3 variant remain unknown.

Material and Methods: We prospectively monitored 83 obese individuals (BMI 35 – 64 kg/m²) before and after bariatric surgery. The PNPLA3 p.I148M variant was genotyped using a fluorescent PCR-based assay. All patients underwent liver biopsy at the moment of surgery. Hepatic steatosis was determined before surgery using three different procedures, i.e. (A) Folch: biochemical determination of hepatic triglyceride contents; (B) semiquantitative histological steatosis grade: 0: no steatosis, 1: 1 – 33%, 2: 34 – 66%, 3: > 66% steatosis; and (C) multi-echo MRI. One year later, steatosis was re-evaluated by MRI and Folch values were estimated with a novel MRI-based equation (Jiménez-Agüero et al. BMC Med 2014).

Results: Overall, 54 (65%) individuals carried the PNPLA3 genotype [II] and 29 (35%) were carriers of at least one prosteatotic allele [M]. The number of individuals carrying this risk allele increased with the grade of steatosis before surgery (grade 0: 25%; 1: 32%; 2: 36%; 3: 42%). Presence of this allele was associated with increased hepatic triglyceride contents (P=0.01), increased alkaline phosphatase activities (P=0.01) and a trend to increased MRI-steatosis (P=0.10). Of note, median weight loss after bariatric surgery was higher in individuals carrying the susceptible PNPLA3 allele [M] as compared to carriers of genotype [II] (45 vs. 37 kg, P<0.01). Accordingly, patients carrying the prosteatotic allele [M] demonstrated a higher decrease of liver fat one year after surgery as compared to individuals with the common genotype, based on both MRI fat fraction and estimated Folch values (P=0.05 and P<0.01, respectively). In regression analysis, the PNPLA3 mutation outscored weight loss as predictor of FLD improvement (P=0.05 vs. P=0.10, respectively).

Conclusions: Obese patients with PNPLA3-associated steatohepatitis show better improvement of hepatic steatosis after bariatric surgery as compared to carriers of PNPLA3 wild-type alleles. Bariatric surgery can reduce the harmful effects conferred by the PNPLA3 mutation and obesity.
IS ASPIRINE USE A PROTECTOR FACTORS OF NONALCOHOLIC FATTY LIVER DISEASE?

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1Internal Medicine, Gastroenterology, University of Medicine and Pharmacy “Nicolae Testemitanu”, 2Interventional Cardiology, Institute of Cardiology, 3Genetic Laboratory, University of Medicine and Pharmacy “Nicolae Testemitanu”, 4Morphopatology, University of Medicine and Pharmacy “Nicolae Testemitanu”, Chisinev, Moldova, Republic of

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Introduction: Nonalcoholic fatty liver disease is now conceptualised as a ‘multiple hit’ process, consisting of insulin resistance, which causes a reversible fat accumulation in hepatocytes as the first hit, and a combination of oxidative stress, lipid peroxidation and pro-inflammatory cytokines as the subsequent hits. Aspirin may affect oxidative stress, vascular inflammation and insulin sensitivity.

Aims: To investigate an association between aspirin use and NAFLD prevalence in the patient with arterial hypertension

Material and Methods: A total of 625 patients (mean age: 48.15 ± 10.5 years, 72% males) who had arterial hypertension and underwent ultrasonography were included in the study; of those, the 167 patients (mean age: 49.62 ± 9.97 years, age range: 23-73, 85/167 – 50.9% females) were identified as having NAFLD and 458 as control. Aspirin use during the month prior to interview was categorised as never use (0 times), occasional use (1–14 times) and regular use (≥15 times).

Results: Patient with NAFLD were more likely to be obese, physically inactive, have diabetes. This group were less frequently use aspirin, than patient from control group. In the multivariate unconditional logistic regression analysis, regular relative to no aspirin use was inversely associated with prevalent NAFLD. Odds ratio = 0.23, 95% confidence interval (CI) 012–0,42; p < 0,01.

Conclusions: Regular aspirin use (≥ 15 times per month) associated with a lower prevalence of NAFLD in case of patient with arterial hypertension and may be had a protective effect in development of nonalcoholic fatty liver disease.

Key words: nonalcoholic fatty liver disease, aspirin, arterial hypertension
POSTERS

ABSTRACTS

SERUM FERRITIN LEVELS EVEN IN NORMAL RANGE PREDICTS HISTOLOGICAL SEVERITY IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE IN INDIA

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Introduction: Recently, hepatic iron overload and its correlation with chronic liver disease have been considered. In the last decade, many studies have found a relationship between hepatic iron and NASH or its progress. Ferritin levels have been implicated in the adaptive response of hepatic endothelial cells to the oxidative stress. Being utilized in western literature as a marker for fibrosis, the levels in Indian patients indicative of fibrosis is not known.

Aims: The aim of the study was to determine the levels of serum ferritin which predict fibrosis in Indian patients with non-alcoholic fatty liver disease and to establish correlation between Fibroscan values and serum ferritin levels.

Material and Methods: The clinical, biochemical, radiologic and histological findings of consecutive adult NAFLD patients accessed at a tertiary care center over a 3-year period were analysed. Those with concurrent liver diseases were excluded. Fifty five patients of 250 NAFLD patients, with fatty liver on ultrasound and raised enzymes (> ULN) underwent liver biopsy. Patients were stratified into two groups based on their histological stage steatosis (with or without inflammation) but no fibrosis and NASH with Fibrosis/cirrhosis. Serum ferritin levels were measured at the same time as getting liver biopsy. Fibroscan was carried out in each of these patients. These were compared with 50 age and sex matched controls with normal ultrasound, liver enzymes and no history of alcohol. Student t test was used as the test for significance.

Results: Fifty five NAFLD patients diagnosed on ultrasound and with raised enzymes underwent biopsy. Steatosis (with or without inflammation, but no fibrosis/ballooning) was seen in 35 patients, fibrosis/ballooning in 14 patients and cirrhosis in 6 patients. Mean ferritin levels in groups with NAFL and NASH were 39.4 and 72.7 ng/ml respectively (p<0.001). The mean ferritin levels in NAFLD and controls were 51.2 and 35.2 ng/ml respectively (p<0.05). The AUC of serum ferritin at value 48.0 ng/ml is 0.779. The coefficient of correlation between Fibroscan and serum ferritin levels was 0.9864 while that with ALT and AST is 0.69. Serum ferritin at the cut-off of 48ng/ml differentiates significantly patients with fibrosis and higher Fibroscan levels.

Conclusions: Serum Ferritin is low in Indian individuals and levels even within apparently normal range indicates fibrosis and cirrhosis. Indians should utilize 48.0 IU/ml as cut-off for fibrosis in NAFLD. Fibroscan correlates well with serum ferritin levels.
PROSPECTIVE COMPARISON OF NONINVASIVE FIBROSIS ASSESSMENT TO PREDICT ADVANCED FIBROSIS OR CIRRHOSIS IN PATIENTS WITH NAFLD

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of liver disease worldwide. Its prevalence continues to rise, and it threatens to become a serious health problem. This study aimed to evaluate the diagnostic accuracy of non-invasive fibrosis assessment to predict advanced fibrosis or cirrhosis in Asian patients with NAFLD.

Aims: This study aimed to evaluate the diagnostic accuracy of non-invasive fibrosis assessment to predict advanced fibrosis or cirrhosis in Asian patients with NAFLD.

Material and Methods: One hundred sixteen patients with a liver biopsy–confirmed diagnosis of NAFLD were prospectively evaluated between March 2013 and September 2014. Liver stiffness measurement (LSM) was performed by acoustic radiation force impulse (ARFI) elastography in all patients. Aspartate aminotransferase to alanine aminotransferase ratio (AAR), Fib-4, aspartate aminotransferase to platelet ratio index (APRI), NAFLD fibrosis score (NFS) and BARD scores were calculated according to published algorithms. Diagnostic measurements of serum fibrosis indices and ARFI imaging were compared to predict advanced fibrosis or cirrhosis by analysing the area under the receiver operating characteristic (AUROC) curve.

Results: The median age of the study population was 54.3 years (range, 18–78). Fib-4, NAFLD fibrosis score, BARD score and LSM showed significant, positive correlations with METAVIR stages (P<0.001). LSM by ARFI had the greatest AUROC for predicting advanced fibrosis (≥F3) (0.883; 95% CI, 0.804–0.961) and cirrhosis (F4) (0.926; 95% CI, 0.848–1.000). And Fib-4 had the good AUROC for predicting cirrhosis (F4) (0.873; 95% CI, 0.803–0.942).

Conclusions: Liver stiffness by ARFI is exhibited best diagnostic performance for predicting advanced fibrosis and cirrhosis in patients with NAFLD. In addition, Fib-4 and NFS are useful non-invasive fibrosis indices for assessment of hepatic fibrosis in patients with NAFLD.
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RANDOMISED PLACEBO-CONTROLLED TRIAL ASSESSING WHETHER PROBIOTIC SUPPLEMENTS AMELIORATE DEPRESSIVE SYMPTOMS IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS INFECTION

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Introduction: Patients with chronic hepatitis C virus (HCV) infection frequently present with co-morbid depression. The pathophysiology of depression is multifactorial, and intestinal microbiota have been reported to influence depressive symptoms. Studies in mice report specific strains of gut bacteria to positively influence brain neurotransmitters implicated in depression, such as GABA. A randomised placebo-controlled trial reported a probiotic supplement to alleviate psychological distress and depressive symptoms in otherwise healthy individuals (Messaoudi et al. Br J Nutr 2011).

Aims: Given the high prevalence of depressive symptoms in patients with chronic HCV infection, this study assesses whether oral administration of select commensal bacteria modulates depressive symptoms and alters intestinal flora architecture in favour of increased lactobacillus and bifidobacterium in these patients. We hypothesised that probiotic supplementation reduces the severity of depressive symptoms.

Material and Methods: A randomised, double-blind placebo-controlled study is being carried out in adults with chronic HCV infection and depression, that have been awaiting new DAA-based antiviral therapies. The intervention product contains two strains of lactic acid bacteria Lactobacillus helveticus and Bifidobacterium longum. Patients are randomly given the probiotic or identical placebo orally for 60 days. Patients are assessed using a validated self-report questionnaire (Beck Depression Inventory-II) for depressive symptoms at baseline, and after 30 and 60 days of treatment. Fecal samples collected during these time points enable us to characterise the microbial community using 16S rRNA pyrosequencing and to assess for changes in the gut microbiota in parallel to changes in depressive symptoms. Body composition using bioelectrical impedance analysis and other potential confounders such as dietary intake and physical activity are captured during all study time points, using food diaries and actigraphs.

ASSESSMENT OF ANTI-INFLAMMATORY AND DIRECT ANTIFIBROTIC EFFECT OF ORAL HEPATOTROPIC DPP4 INHIBITORS IN MODELS OF NASH AND BILARY FIBROSIS

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Introduction: Non-alcoholic steatohepatitis (NASH) is characterized by steatosis, lobular inflammation and progressive parenchymal fibrosis. Glucagon like peptide 1 (GLP-1) is an attractive molecule for the treatment of insulin resistance. GLP-1 is rapidly inactivated by cell surface dipeptidyl peptidase-4 (DPP-4).

Aims: Therefore we studied the effect of two indirect GLP-1 agonists, hepatotropic DPP4-inhibitors, on liver inflammation and fibrosis in models of NASH and biliary fibrosis.

Material and Methods: Linagliptin and Sitagliptin were administered daily by oral gavage to Mdr2KO mice and to C57BL/6mice fed a methionine and choline deficient (MCD) diet for 6 weeks. Hepatic fibrosis was assessed by morphometric analysis of Sirius red stained collagen and measurement of hydroxyproline content. Hepatic inflammation and fibrosis were assessed by semiquantitative immunohistochemistry. Fibrosis and inflammation related transcript levels were measured by quantitative real-time polymerase chain reaction (qPCR). Ex vivo analysis of hepatic inflammatory cells was done by FACS.

Results: In the MCD model, both Linagliptin and Sitagliptin lowered serum ALT, AST, ALP and LDH and also decreased fibrosis and inflammation related gene expression (αSMA, procollagen α1(I), TIMP-1, TGFβ1, MMP9, MMP-13, CD68, CCL3 and TNF α) compared to vehicle-treated controls. The expression of collagen area (Sirius red), αSMA, procollagen type III, CD68, F4/80, Caspase 3, as determined by semiquantitative immunohistochemistry, was significantly suppressed.
There was a decrease of hepatic CD11b\(^+\)Ly6C\(^{\text{high}}\) (proinflammatory monocytes/macrophages) and of total F4/80\(^+\) cells (macrophages, Kupffer cells) when compared to mice that received the MCD diet without treatment. Linagliptin and Sitagliptin treatment lead to a significantly attenuated hepatic fat accumulation and a reduction of transcript levels of SREBP-1c, FAS, adiponectin and LPL vs untreated disease controls. In Mdr2KO mice 10 and 50mg/kg/day of Linagliptin significantly decreased procollagen \(\alpha_1(I)\), TGF\(\beta_1\), TIMP-1, MMP-8 transcript levels, but increased putatively anti-fibrotic MMP-9 and -13.

**Conclusions:** In mice fed the MCD diet for 6 weeks, the DPPIV inhibitors significantly decreased inflammation and apoptosis. Linagliptin and Sitagliptin mildly decreased liver injury and fibrosis through inhibition of inflammatory and apoptosis pathways that are prevalent in human NASH. However, efficacy on fibrosis is likely attenuated by divergent effects on fibrolysis.
RESVERATROL IMPROVES THE PATHOGENESIS OF NASH/NAFLD VIA THE INHIBITION OF ENDOTOXIN-INDUCED LIVER INFLAMMATION AND FIBROSIS

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Introduction: Nonalcoholic fatty liver disease (NAFLD) morbidity rate in Asia Pacific region is close to 12–24%. In spite of its high prevalence, there is no pharmacologic therapy. Resveratrol (RSV), which inhibits activation of STAT3, is known to improve the pathogenesis of steatosis or steatohepatitis in murine model. However, Veronique S recently reported that RSV did not significantly improve any features of NAFLD patients. We previously reported that overexpression of CD14 via activation of leptin-STAT3 signalling induced hyper-inflammatory response, resulting in progression from simple steatosis to steatohepatitis with liver fibrosis and soluble CD14 levels reflect liver inflammation in patients with nonalcoholic steatohepatitis.

Aims: The aim of this study was to investigate whether RSV improves the pathogenesis of steatosis or steatohepatitis in murine model with serum CD14 high value.

Material and Methods: Eight-week-old male C57BL/6J mice were randomly distributed into 3 groups of 10 animals each: a high fat diet group (HF), HF supplemented with 2mg/kg RSV daily (HFR2), and HF supplemented with 20mg/kg RSV daily (HFR20). After 12 weeks of dietary treatment, the mice were euthanized and relevant tissues were prepared for subsequent analysis. In this study, E. coli-derived LPS (0.25 mg/kg) was used.

Results: HF showed high value of serum CD14, compared with HFR. RSV prevented the high fat–induced steatosis assessed by semiquantitative grading, which furthermore corresponded with a complete normalization of the hepatic triglyceride content (P<.001), despite no change in total body fat, and hepatic SREBP1c expression was significantly decreased as compared with HF. HFR showed significant inhibition of hepatic CD14 expression through suppression of STAT3 activity in Kupffer cells, following inhibition of a single low-dose LPS-induced liver damage. Moreover, long-term low-dose LPS-induced liver fibrosis in HFR is significantly decreased as compared with HF.

Conclusions: These data indicated that RSV improves not only the pathogenesis of steatosis thorough inhibition of lipogenesis but also steatohepatitis through inhibition of ET-induced liver damage via suppression of STAT3-CD14 signalling in Kupffer cells. The RSV may have application for the treatment of NAFLD patients with serum sCD14 of high value.
MICRO RNA-27B PROMOTES NONALCOHOLIC STEATOHEPATITIS THROUGH THE INHIBITION OF PPARα AND MTTP IN VIVO ANIMAL STUDY.

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Introduction: MicroRNAs (miRs) are a class of small non-coding RNAs that function to control gene expression by inducing the degradation or inhibiting the translation of mRNA through an association with its 3'-untranslated region (3'UTR). The involvement of miRs on the onset of nonalcoholic steatohepatitis (NASH) is unknown. We found that expression of miR-27b (27b) increased in liver biopsy specimens of NASH patients compared with that of controls subjects by microarray analysis.

Aims: The aim of this study was to elucidate the role of hepatic 27b in the pathogenesis of NASH in murine model.

Material and Methods: Five-week-old male C57BL/6J mice were randomized into 4 groups (n=16 mice): basal diet (BD)-fed control mimic (BD-Con, n=4), BD-fed miR-27b-mimic (BD-27b, n=4), high fat diet (HFD)-fed control mimic (HFD-con, n=4), HFD-fed 27b-mimics (HFD-27b, n=4). In this study, 27b mimics or vehicle is injected intravenously.

Results: Comparing the HFD-con, liver weight of HFD-27b was significantly increased. Pathological findings revealed the marked progression of steatosis, inflammatory cell infiltration and fibrosis. Transfection of miR-27b-mimic significantly inhibited the expression of the hepatic microsomal triglyceride transfer protein (MTTP) which is the key enzyme for the secretion of VLDL and peroxisome proliferator-activated receptor alpha (PPARα), suggesting that upregulation of 27b promotes the accumulation of triglyceride.

Conclusions: 27b controls multiple gene levels that are involved in hepatic fat accumulation, and increased expression of mir27b might promote the steatohepatitis under the high-fat condition. This is the first report that application of 27b in vivo murine model could cause the NASH condition similar to that of patients.
PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE HAVE SIMILAR GENERAL HEALTH PERCEPTIONS AS THOSE OF HEALTHY CONTROLS

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Introduction: Although patients with non-alcoholic fatty liver disease (NAFLD) are generally asymptomatic, they report both physical and emotional lower quality of life and over-utilize medical services as compared to healthy controls. Fatigue and lack of confidence to exercise have been described in NAFLD patients.

Aims: The study aim was to examine the association between NAFLD and general health perception.

Material and Methods: A cross-sectional study in 213 subjects, with no known liver disease or history of alcohol abuse. The evaluation included: self-reported general health perception, physical activity habits, frequency of physician’s visits, fatigue impact scale and abdominal ultrasound.

Results: There were 54% males, mean age 58±9.6 years and 70/213 had NAFLD. All anthropometric measures, liver enzymes and fasting glucose blood levels were significantly higher among NAFLD as compared to controls (age and gender were comparable). Reduced motivation for activity with physical effort (27.1% vs. 33.6%, P=0.343) and actual need for reduced activity (20% vs. 19.6%, P=0.781) as well as other parameters in the fatigue impact scale were equivalent between the NAFLD and the control groups. Time spent in leisure time physical activity was lower among the NAFLD subjects, however, fatigue and fear of physical harm as explanations for lack of physical activity were evenly reported between the groups (P=0.436). Self-reported general health perception status did not differ between NAFLD and control groups. In a multivariate analysis, NAFLD was not associated with a lower self-reported general health perception (OR=0.735, 95% CI 0.349-1.548, P=0.417). Nevertheless, the odds for “very good” self-reported general health perception decreased with increasing level of BMI and age, and increased among males and those that exercised regularly. In terms of health service utilization, there was a significant difference in the reasons for family doctor visits (P=0.016); NAFLD patients had more acute disease visits while controls had more routine check-ups and chronic diseases visits.

Conclusions: Patients with NAFLD were comparable to controls in terms of fatigue and self-reported general health perception. Health service utilization due to chronic diseases was less common among the NAFLD group. Our results imply that fatty liver without clinically significant liver disease has a small impact on health perception.
THE ASSOCIATION BETWEEN SERUM LEVELS OF URIC ACID AND ALANINE AMINOTRANSFERASE IN A POPULATION-BASED COHORT

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Introduction: Elevated serum uric acid is frequently observed in patients with the metabolic syndrome. A strong correlation exists between this syndrome and nonalcoholic fatty liver disease (NAFLD).

Aims: We aimed to test the association between uric acid and elevated alanine aminotransferase (ALT) in a large population-based cohort.

Material and Methods: A cross-sectional study using real-world data from a large public health organization in Israel (Maccabi Healthcare System). The population consisted of individuals aged 20-60 years old who underwent blood tests for ALT and uric acid for any reason during 1997-2012. Individuals with secondary liver disease, celiac and inflammatory bowel-disease were excluded. Subgroup analysis was performed in subjects who were diagnosed with fatty liver in the medical records (n=2,628). This database includes medical history, diagnoses, patient consultations, prescription drug purchase, laboratory and imaging tests.

Results: The study population included 82,608 people (32.5% men, mean age 43.91±10.15 years). Subjects in the upper quartiles (>5.6 mg/dL) of uric acid level were significantly (P<0.001) more likely to have a poorer metabolic profile in terms of glucose, serum lipids, diabetes and hypertension. Categorizing serum uric acid into deciles demonstrated a significant positive dose-response association with the rate of elevated serum ALT (P for trend<0.001). In multivariate logistic regression analysis, controlling for potential confounders, the association between uric acid and elevated ALT persisted both in the total population (OR= 1.18, 1.10-1.28 95% CI, OR=1.49, 1.38-1.62, OR=2.10, 1.93-2.29) for the 2nd, 3rd and 4th quartiles respectively compared to the 1st) and in the subgroup of subjects who were diagnosed with fatty liver (OR= 1.77, 1.22-2.57, for the 4th quartile compared to the 1st). With stratification by gender or BMI categories, the association between uric acid and elevated ALT was maintained in all categories.

Conclusions: Serum uric acid is independently associated with elevated ALT, as a surrogate for NAFLD, and thus may serve as a serum marker and should be further investigated as a risk factor for NAFLD.
MORPHOLOGICAL AND FUNCTIONAL ASSESSMENT CRITERIA OF LIVER SINUSOIDAL ENDOTHELIAL DYSFUNCTION IN EXPERIMENTAL HEPATIC STEATOSIS

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Introduction: Endothelium of the sinusoids of the liver have a significant role in regulating the processes of local hemostasis, proliferation, migration of blood cells in the vascular wall and regulation of vascular tone.

Aims: The aims of research - to identify the most significant morphological and functional criteria of endothelial dysfunction (using complex morpho-functional approach) in the sinusoids of the liver in experimental hepatic steatosis.

Material and Methods: Research was conducted on 40 white adult male rats. The animals were divided into 2 groups: control - 10 animals were fed with a standard semi-synthetic starch-casein diet and research - 30 rats, which was held on high fat diet for 6 weeks to create a model of hepatic steatosis.

Results: Histological examination of the liver revealed lipid accumulation in hepatocytes like large droplets mostly in the area of the central vein in rats with experimental steatosis. Endothelial layer in arterioles was not integrated, but contained areas of endothelial desquamation and foci of proliferation. Restructuring of blood vessels of the liver was characterized by thickening of their walls, narrowing the lumen. Destruction of endothelial barrier was revealed by microscopy. The basal layer of the membrane was damaged, which manifested itself in its irregular thickening and stratification. Much of sinusoidal endothelial cells of capillaries were in a state of apoptosis with condensed chromatin, the remaining corpuscles in a nucleus place and homogenized, fragmented cytoplasm. Abnormal communication with penetrating of red blood cells, white blood cells and detritus were found between dilated Disse spaces. Kupffer cells with the cytoplasm filled with parts of detritus migrated to the area of capillary wall deformation.

Conclusions: Thus, changes in the structure of the endothelium in experimental steatosis in rats with obesity were accompanied by signs of stasis, thrombosis, thickening of vessel walls, increasing the permeability ratio of intima-media edema, reducing the size of nuclei. Endothelium is like the mediator between the blood and other entities of vessels, it becomes a party to inflammatory processes occurring in the liver.
DELETION OF THIOREDOXIN-INTERACTING PROTEIN (TXNIP) PROTECTS AGAINST HIGH FAT DIET INDUCED STEATOHEPATITIS AND LIVER INJURY

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome. Oxidative stress and inflammation play a pivotal role in the pathogenesis of NAFLD. Inflammasomes are shown to be implicated in liver damage, steatosis, inflammation and fibrosis. Nevertheless, TXNIP-mediated activation of Nod-like receptor protein 3 (NLRP3) inflammasome and its involvement in the pathogenesis of HFD-induced NAFLD and non-alcoholic steatohepatitis (NASH) remains unknown.

Aims: In the current study we investigated the role of TXNIP in steatohepatitis and fibrosis.

Material and Methods: Western blotting was used to examine the changes in the protein levels of TXNIP, NLRP3, caspase-1 and IL1β in liver samples from wild type normal diet (WTND), wild type high fat diet (WTHFD), knockout normal diet (TKOND) and knockout high fat diet (TKOHFD) groups. Furthermore, we examined the effect of TXNIP deletion on the development of steatohepatitis and fibrosis by histological as well as immunohistochemical staining using anti-interleukin1 beta (anti- IL1β) and anti- alpha smooth muscle actin (Anti-αSMA) antibodies. All metabolic parameters including glucose intolerance and plasma levels of total cholesterol and triglycerides were also measured after 8-weeks from the start of the research.

Results: Massive hepatic steatosis and prominent lobular and portal inflammation were observed in WTHFD group. Furthermore, an increase in area and percentage area of fibrosis was observed in specimen isolated from WTHFD by 40 folds (n=6; p<0.05).
On the other hand, liver specimen from TKOHFD showed a significantly reduced amount of fibrous tissue being noted around central veins and portal tracts and no fibrous tissue noted in sinusoidal wall after 8 weeks (n=6; p<0.05). Such observed reduction in the accumulation of fibrous tissue together with a concomitant reduction in the expression of IL1b, NLRP3, Caspase-1 and TXNIP in TKOHFD liver samples strongly support the important effect of deletion of TXNIP on accumulation of ECM and inhibition of progression of liver fibrosis.

**Conclusions:** TXNIP deletion can protect against HFD-induced steatohepatitis and its associated pro-inflammatory and fibrotic response through the activation of the NLRP3 inflammasome and its downstream proinflammatory cytokine IL-1β. Therefore, TXNIP signalling system is a potential therapeutic target to treat NAFLD.
INVOLVEMENT OF MERTK RECEPTOR TYROSINE KINASE IN THE HEPATIC FIBROSIS PROCESS.

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Introduction: The major cell type involved in the liver fibrosis is the activated hepatic stellate cells (HSC). Following liver damage, HSC undergo a process of phenotypic transition, known as ‘activation’, leading to increased proliferation and migration, a shift towards production of fibrillar matrix components, and increased expression of pro-inflammatory cytokines. MERTK is a receptor tyrosine kinase with oncogenic properties that is often overexpressed or activated in various malignancies. MERTK exerts a strong antiapoptotic and prosurvival function activating both ERK1/2 and Akt proteins. Several studies reported that protein mediates signalling pathways that lead to antiinflammatory cytokine production as well as enhanced proliferation, migration and invasion.

Aims: MERTK variants have been associated with fibrosis severity at genome wide level in chronic hepatitis C, in particular the variant rs4374383 G>A of MERTK gene has been associated with a reduced liver fibrosis progression, but currently there is no experimental evidences on the involvement of MERTK in HSC modulation and in the hepatic fibrosis process.

Material and Methods: Primary Human HSC were isolated from human livers and cultured on plastic. Cell migration was evaluated in modified Boyden Chambers. C57BL6/J mice were treated with CCl4 (0,5 ml/Kg twice I.P. in olive oil) for 6 weeks to induce liver fibrosis. Balb/C mice were fed with a methionine choline-deficient (MCD) diet for 8 weeks. Intrahepatic gene expression was assayed by quantitative real time PCR

Results: The analysis by immuoprecipitation showed that MERTK is expressed in human HSC. Stimulation of Human HSC with the MERTK ligand GAS6 resulted in a time-dependent activation of ERK1/2. Concerning the phenotypic changes induced by MERTK stimulation, the main effect that we observed was an enhancement of cell migration of Human HSC comparable with the increase in cell motility induced 10 % FBS treatment. In two well-established experimental murine models of fibrosis (Chronic CCl4 and MCD diet). In both models we observed a significant increase of expression of MERTK in treated mice compared to control group. Finally analysis of the MERTK level in liver specimens from patients affected with NASH, presenting different degree of liver fibrosis, shown that MERTK expression correlates with the score of fibrosis observed in NASH patients

Conclusions: In vitro and in vivo data of this study indicate that MERTK could play an important role of in the liver fibrosis process.
LYSOPHOSPHATIDYLCHOLINE (LPC) AS CENTRAL PLAYER FOR HEPATIC FAT ACCUMULATION AND INFLAMMATION: IMPLICATION FOR PATHOGENESIS OF NASH

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Introduction: In NASH the intracellular ratio of phosphatidylcholine (PC): lysophosphatidylcholine (LPC) is decreased due to activation of the membrane localized phospholipase A2 (iPLA₂β).

Aims: Here we aim the evaluation of LPC levels in development and reversal of fat accumulation and inflammation.

Material and Methods: In HepG2 cells the bile acid-phospholipid conjugate ursodeoxycholate-lysophosphatidylethanolamide (UDCA-LPE) as iPLA₂β inhibitor was used to modify intracellular LPC levels. We examined the impact of LPC on JNK1-p and transcription of the heterotetrameric fatty acid transport complex constituted of CD36, FABP₇, caveolin1 and iPLA₂β. A NASH phenotype was generated by exposure of HepG2 cells with high concentrations of oleate bound to albumin (4:1) for 3 h.

Results: Addition of 1 – 10 µM LPC to delipidated cytosolic extracts revealed a dose dependent increase of JNK1-p a central promoter of fatty acid metabolism and lipoapoptosis. In vitro transcription with native HepG2 nuclear extracts exposed to these LPC conditioned cytosolic samples resulted in synthesis stimulation of all members of the fatty acid uptake complex. In contrast, the NASH phenotype was reversed or prevented by incubation with the iPLA₂β inhibitor UDCA-LPE (1 h, 100 µM) suppressing cytosolic LPC levels. Accordingly JNK1-p and transcription of fatty acid transporter biosynthesis were reduced, concomitantly with disappearance of lipid droplets, triglyceride accumulation and LDH release in the medium.

Conclusions: iPLA₂β mediated generation of LPC represents a central regulator for hepatic steatosis and inflammation via JNK1-p. Inhibition of iPLA₂β by nontoxic UDCA-LPE could be an ideal therapeutic strategy against NASH.
SUPPLEMENTATION WITH AMINO ACIDS PROTECTS MICE FROM THE ONSET OF FRUCTOSE-INDUCED NAFLD: BENEFICIAL EFFECTS OF ARGinine AND CIRTrulline ARE ASSOCIATED WITH MODIFICATIONS OF INTESTINAL PERMEABILITY

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Introduction: The prevalence of non-alcoholic fatty liver disease (NAFLD), one of the most frequent chronic liver disease worldwide, continues to increase. Within Europe approximately 3-10% of the general adult population is affected by the disease by now. Several possible risk factors such as genetics, “Western” lifestyle and nutrition but also disturbances of the gut barrier function are discussed to be critical in the onset and progression of NAFLD. Human and animals based studies indicate a role of the metabolism of arginine and/ or citrulline and the development of liver diseases of different entities.

Aims: The aim of the present study was to investigate the effect of an oral supplementation of arginine and citrulline, respectively on the development of Western-style diet (WSD) -induced NAFLD in mice.

Material and Methods: C57BL/6J mice (n = 8) per group were pair-fed with a fructose-enriched WSD or a respective control diet +/- arginine (2.49 g/kg b.w.) or citrulline (2.5 g/kg b.w.) for 6 weeks. Body weight, liver weight, indices of liver damage and markers of glucose metabolism as well as gut barrier function were determined.

Results: As expected, mice developed a liver steatosis with beginning inflammatory changes when being fed a WSD for 6 weeks. In mice concomitantly supplemented with the two amino acids while being fed the WSD, signs of hepatic inflammation i.e. number of neutrophils and TNF (tumor necrosis factor) α protein levels were significantly lower than in mice only fed the WSD.
Number of fatty hepatocytes was similar between the two WSD groups; however, while in mice only fed a WSD macrovesicular fat droplet were predominate in those supplemented with the amino acids microvesicular steatosis was more frequent. The protective effect of an oral supplementation of citrulline and arginine was associated with a protection against the loss of the tight junction protein occludin in the duodenum found in mice only fed a WSD. Additionally, mice fed a WSD and citrulline had lower portal blood endotoxin levels.

**Conclusions:** Our results suggest that citrulline and arginine protect mice from the development of NAFLD through mechanisms involving a decreased translocation of bacterial endotoxin and improvement of the gut barrier. (funded by Deutsche Forschungsgemeinschaft DFG (BE 2376/6-1)
LOSS OF LIPOPOLYSACHARIDE-BINDING PROTEIN (LBP) ATTENUATES DEVELOPMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE IN MICE

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**Introduction:** Results of several human and animals based studies suggest that not only a general overnutrition but also a diet rich in fat, cholesterol and sugar may be critically associated with the development of non-alcoholic fatty liver disease (NAFLD). It also has been suggested that an increased translocation of bacterial lipopolysaccharide (LPS) from intestine into the portal vein and subsequently an activation of Toll-like receptor (TLR) -dependent signalling pathways in the liver may contribute to the development of NAFLD.

**Aims:** The aim of the present study was to investigate if the loss of lipopolysaccharide binding protein (LBP) protects mice from the development of NAFLD.

**Material and Methods:** Eight weeks old LBP -/- and +/+ mice were pair-fed with either a diet rich in fat, cholesterol and fructose- (=‘Western-style’ liquid diet, WSD) or a control liquid diet (n = 7-8 per group). Parameters of liver injury, number of F4/80 positive cells, expression of TLR-4, MyD88 (myeloid differentiation primary response gene 88) and concentration of 4-HNE (4-hydroxynonenal) as well as iNOS (inducible nitric oxide synthase) were determined in liver tissue.

**Results:** Despite similar total caloric intake, weight gain and liver to body weight ratio indices of liver damage NAFLD as determined by liver histology and transaminases were markedly lower in LBP -/- mice fed the WSD than wild-type animals. In line with these findings number of neutrophils but also F4/80 positive cells and iNOS protein levels were also only significantly increased in wild-type mice fed the WSD whereas in livers of LBP-/- mice fed the WSD these markers were almost at the level of controls. Similar differences were also found by the mRNA expression of TLR-4 and MyD88 as well as content of 4-HNE in the liver.

**Conclusions:** Our results so far indicated that LBP could be a critical factor in the development of NAFLD. (Funded by the BMBF, FKZ: 01GI1122H (IB / CT) as part of the competence network obesity)
FATTY LIVER PRESERVATION AND AUTOFLUORESCENCE REAL TIME MONITORING OF OXIDATIVE DAMAGE

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Introduction: The risk of disease progression or organ dysfunction during surgery and transplantation of fatty livers is increased by oxidative stress. A rat liver model of NASH (induced with methionine/choline-deficient diet-MCD) exhibited a general subversion of metabolism reflecting in alterations of autofluorescence (AF) emission properties. These consisted in NAD(P)H and flavin AF signal changes, ascribable to redox metabolism alterations, and rising in vitamin A, protein, and lipopigment AF contribution.

Aims: Since lipopigments derive from the oxidation of unsaturated lipids, fluorescing fatty acids and their oxidized products were investigated in isolated MCD livers under different preservation conditions as early AF biomarkers of oxidative alterations.

Material and Methods: Control and 2 week MCD diet livers were isolated and submitted to 6-h Cold Storage (CS) or subnormotermic Machine Perfusion (MP) preservation, followed by reperfusion at 37°C with oxygenated medium. In vivo AF analysis -exc 366nm- was performed via fiber optic probe. Each endogenous fluorophore contribution to the overall AF emission was estimated through spectral fitting procedure, similarly to in situ biochemical analysis. Tissue oxidative stress (TBARS, and GSH) and mitochondrial dysfunction (ATP/ADP) were assayed with conventional methods.

Results: Liver AF emission amplitude was influenced to different degree by CS and MP preservation and subsequent reperfusion, consistently with a MCD liver mitochondrial dysfunction affecting the response to oxygen availability and temperature, in terms of NAD(P)H/flavin redox changes. Both controls and MCD diet livers showed spectral shape alterations indicating a rising in oxidized lipids after preservation, in a good correlation with TBARS. The phenomenon more marked in MCD than in control livers indicated a strong influence of oxidative stress, independently from CS or MP preservation. To note that fluorescing fatty acids were not depleted, consistently with dynamic equilibria in liver lipid pool composition.

Conclusions: The in vivo, real time AF analysis was validated to evidence the rising of oxidative effects induced by external stimuli even in a model of mild metabolic alterations. These results provide further support in AF experimental applications to assess drug response and toxicity, and in the set-up of innovative organ preservation strategies. (Supported by Fondazione Cariplo, grant n° 2011-0439).
CHANGES IN SERUM LEVELS OF ASYMMETRIC-DYMETHYLARGININE (ADMA) IN A RAT MODEL OF NAFLD: ROLE OF CATIONIC TRANSPORTERS

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Introduction: The liver plays a crucial role in the metabolism of asymmetric-dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthase. ADMA is synthetized by protein methyltransferases (PRMTs), metabolized via dimethylarginine-dimethylaminohydrolase (DDAH) and transported across cell membrane by cationic amino-acid transporters (CATs).

Aims: This study investigated whether, in a rat model of non-alcoholic fatty liver disease (NAFLD) changes in serum levels of ADMA occur and the mechanisms involved.

Material and Methods: Male Wistar rats underwent to NAFLD induced by 4 weeks of feeding with an MCD diet (methionine/choline-deficient diet). Blood samples and hepatic biopsies were collected after 1, 2, 3, and 4 weeks. Serum hepatic enzymes (AST, ALT and Alkaline Phosphatase), total and direct bilirubin and ADMA were evaluated. Hepatic biopsies were used for in situ NAD(P)H autofluorescence detection and for mRNA expression of PRMT-1, DDAH-1 and ADMA transporters (CAT-1, CAT-2A and CAT-2B) by RT-PCR. Tissue DDAH activity and content of lipid peroxides, glutathione and ATP were also quantified.

Results: NAFLD injury was confirmed by altered serum levels of hepatic enzymes. A marked increase in total and direct bilirubin was detected. A time-dependent decrease in serum ADMA levels and mRNA expression of CAT-2A and CAT-2B was obtained. On the contrary, an increase in mRNA expression of DDAH-1, PRMT-1 and CAT-1 was found. The hepatic DDAH activity decreased with a concomitant increase in oxidative stress, as demonstrated by high lipid peroxide levels and low GSH content. A decrease in ATP levels and in the NAD(P)H\text{bound/free} ratio reflecting the mitochondria alterations was also detected in MCD rats.

Conclusions: These results indicate that while an increase in DDAH mRNA was found, the oxidative stress observed can contribute to the reduction of DDAH activity. This enzyme is a cysteine hydrolase that may be inhibited by increased reactive oxygen species associated to mitochondria dysfunction. The observed decrease in serum ADMA found using an animal model of NAFLD may be due to the increase in the ADMA transporter CAT-1. These data confirm and support the crucial role of the liver in the control of ADMA levels by taking up large amounts of ADMA from the systemic circulation. (Supported by Fondazione Cariplo, grant n° 2011-0439).
PATHOGENESIS OF NAFLD: MITOCHONDRIA DAMAGE, OXIDATIVE STRESS AND MATRIX METALLOPROTEASE ACTIVATION IN TWO ANIMAL MODELS OF HEPATIC STEATOSIS

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Introduction: Animal models of hepatic steatosis have allowed the understanding of mechanisms involved in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). By comparing these models, a working hypothesis for NAFLD pathogenesis emerges as well as the mechanism implicated in the progress to non-alcoholic steatohepatitis (NASH).

Aims: This study investigated the oxidative stress, mitochondria damage and matrix metalloprotease activation in fatty livers using two rat models of NAFLD such as the methionine and choline deficient (MCD) diet model and obese fa/fa rats.

Material and Methods: Male Wistar rats underwent to NAFLD induced by 3-week MCD diet; blood samples and hepatic biopsies were collected up to 3 weeks. 12-week male old obese (fa/fa) and lean (fa/-) male Zucker rats were also used. Serum levels of hepatic enzymes (AST, ALT, Alkaline Phosphatase), total and direct bilirubin were quantified. In addition, the serum levels of cholinesterase, enzyme synthesized by hepatocytes and recently reported as index of liver injury, was assessed (Boeykens et al., PLoS One 2013). Tissue glutathione (GSH), lipid peroxides, ATP/ADP ratio and matrix metalloprotease activation (MMP-2, MMP-9) were also evaluated.

Results: No significant changes in serum AST, ALT and Alkaline Phosphatase were found comparing the two animal models. A marked serum increase in total and direct bilirubin in 3-weeks MCD rats was detected. A significant increase in serum cholinesterase was found in both animal models but in obese fa/fa rats was higher when compared with MCD rats. Mostly decrease in tissue GSH levels and increase in the lipid peroxides were found in the MCD group. A better mitochondrial function, as documented by the ATP/ADP ratio and no MMP-9 and poor MMP-2 activation, was measured in obese fa/fa rats when compared with the MCD animals. Instead, in MCD rats a significant increase of MMP-2 activation occurred.

Conclusions: MCD rats exhibit a marked oxidative stress and mitochondria damage concomitant with an MMP-2 activation, when compared with obese fa/fa rats. Our results support the hypothesis that the reported spontaneous development to severe NASH, observed only in MCD rats and not in obese fa/fa animals, might be associated to these events already detectable in the early period of treatment. (Supported by Fondazione Cariplo, grant n° 2011-0439).
THE COMBINATION OF PROBIOTICS AND PREBIOTICS SUPPLEMENTATION IMPROVES LIPID METABOLISM, NAFLD AND OBESITY IN OB/OB MICE

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Introduction: Recent evidences suggest that changes in gut microbiota could modulate the intestinal flora and improve metabolic-related disorders, including nonalcoholic fatty liver disease (NAFLD). The reduction of pro-inflammatory cytokines, the improvement of the immune system, reduction of intestinal infections, could increase lean mass and decrease fat mass.

Aims: The aim of this study was to examine the effects of a supplementation with probiotics and prebiotics in NAFLD and obesity in ob/ob mice.

Material and Methods: Ob/ob male mice, weighting 40g, received in drinking water a combination of probiotics and prebiotics (L.acidophilus, L.rhamnosus, L.paracasei, B.lactis and Frutoolygosacaride) for 8 weeks (Treated group; n=8). Control group (n=6) received only drinking water. All animals received standard diet. After 8 weeks, the difference between the initial and final body weight was calculated in all animals and liver tissues were collected for mRNA and miRNA isolation, and histological analysis. Genes related to lipids metabolism (SREBP1C, miR-33a, MTP, PPAR-γ), mitochondrial oxidation (CPT, PPAR-α) and inflammatory response (TL4, TL9, IL22RA, NFKB) were evaluated by RT-qPCR.

Results: The combination of probiotics and prebiotics supplementation improved liver histology, and decreased visceral fat mass and weight gain, reducing obesity in comparison to control group. Our study showed a statistically significant decrease in MTP (p=0.001), PPAR-γ (p=0.023), PPAR-α (p=0.016) and CPT (p= 0.010) mRNA expression and increased expression of miR-33a (0.5 fold change) in treated group compared to control. However, there were no relevant modifications in SREBP1C, TL4, TL9, IL22RA and NFKB.

Conclusions: The combination of probiotics and prebiotics improved liver histology, decreased visceral fat mass and reduced obesity, and modulated genes involved in the synthesis, exportation and oxidation of hepatic lipids, as well. However, we cannot rule out that other mechanisms related to inflammatory pathways could contribute to the improvement of NAFLD.
THE PRESENCE OF WHITE MATTER LESIONS IS NOT ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE BUT WITH ITS HISTOLOGICAL SEVERITY

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Introduction: Nonalcoholic fatty liver disease (NAFLD) has been associated with increased cardiovascular risk, including coronary artery disease and cerebrovascular events. No studies however assessed the potential relationship between NAFLD and subclinical cerebrovascular alterations.

Aims: We tested the correlation between NAFLD and its histological severity with vascular white matter lesions (WML) in patients with biopsy-proven NAFLD and in non steatosic controls.

Material and Methods: The anthropometric, biochemical and metabolic features were recorded in 77 consecutive biopsy-proven NAFLD (Kleiner score), and in 35 controls with normal ALT, without chronic liver diseases, and without ultrasonographic evidence of steatosis. All patients underwent minimental test (MMT) and magnetic resonance assessment of WML. MMT was considered pathologic if \(<23\). WML were classified according to the Fazekas score in absent (0/III), or present (mild I/III; moderate II/III, and severe I/III). For purpose of analyses all controls, as plausible, were considered without NASH and without F2-F4 liver fibrosis.

Results: WML were found in 26% of the entire cohort (29/112), even if of a moderate-severe grade in 5 patients only. The prevalence of WML was similar in NAFLD compared to no NAFLD (27% VS 23%; \(p=0.62\)).
Age ≥50 yrs, female gender, type 2 diabetes, arterial hypertension, presence of NASH (35% vs 18%, p=0.05) and presence of F2-F4 fibrosis (43% vs 17%, p=0.003) were associated with WML presence (p=<0.01). At multivariate analysis age >50 yrs (OR 3.44 95% CI 1.01-11.6. p 0.04), female gender (OR 3.71. 95% CI 1.28-10.7. p 0.01), and F2-F4 fibrosis (OR 3.39. 95% CI 1.17-9.84. p 0.02) were maintained as factors independently associated with WML. When considering NAFLD patients only, we confirmed F2-F4 fibrosis as the only independent predictor of WML (OR 4.24. 95% CI 1.14-15.7. p 0.03). A pathological MMT was found in 10/112 patients (9%) - all of them with NAFLD. Specifically the prevalence of an altered MMT was 17% in patients with WML and 8% in those without.

Conclusions: The presence of WML is not associated with NAFLD but with its histological severity. Clinical implications of this issue need to be assessed by longitudinal studies. The ability of MMT to detect subclinical WML was poor.
PILOT STUDY OF A NEW TREATMENT IN NAFLD/NASH INTERFERING INTESTINAL MICROBIOTA AND BILE ACIDS RESORPTION AND METABOLISM

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Introduction: Intestinal microbiota plays an important role in modulating inflammation and absorption of bile acids in the enterohepatic circuit, thus a dysfunction might trigger the NAFLD/NASH. Rifaximine (RFX) is known to eradicate intestinal bacterial overgrowth and followed by a symbiotic (SYN) that increases colonization with Bifidobacteria exert an anti-oxidant and immunomodulatory effect. Ursodeoxicolic acid (UDCA) has been used in previous studies in NASH.

Aims: To assess the efficacy of a new combined therapy consisting in RFX, followed by a SYN (prebiotic+probiotic+ vitamins) and associated to UDCA in a discontinued regimen for improving NASH.

Material and Methods: 40 patients diagnosed with NASH on transaminases (AST/ALT) elevation, US, serological tests for NASH/fibrosis and histology, were prospectively randomized between Jan.2012-Sept. 2014 into 2 similar groups: 20 patients were treated with RFX 1200 mg/day 10 days, followed by SYN 1/day, 10 days, in association with UDCA 15 mg/Kg/day; 20 patients received only UDCA. This regimen was repeated for 3 consecutive months, 3 times in 1 year, with evaluation after 1 year with regard to the weight loss, AST/ALT ratio, insulin resistance, steatosis and inflammation grade.

Results: Both groups were comparable: mean age was 45 to 47 years, more men; 16/20 in gr. 1 and 15/20 in gr.2 were overweight, 90% with abdominal obesity, 40% from gr.1 and 50% in gr.2 had diabetes mellitus, 50% with insulin resistance, 80% and 70% had hyperlipidemia, 30% vs. 50% had HTA. 90% over 80% had AST/ALT ratio>1. NASH test was positive in all patients, on liver biopsies steatosis was over 60%, inflammation grade 2 to 3 and fibrosis grade 2, no liver cirrhosis. Associated diet, change in life style, medication to correct the co-morbidities had a compliance of about 60%. After 1 year of follow-up improvements were found with regard to: weight loss of 4-10% in 70% of cases in both groups, normalization of ALT, AST and insulin sensitivity in 90% from gr. 1, vs. 40% in gr.2, decrease in steatosis infiltration by 40% and in fibrosis by1 stage only in gr.1, with no side effects.

Conclusions: Several cycles of an associated regimen with RFX+SYN+UDCA, with diet measures and weight loss, improved significantly liver histology, steatosis and inflammation in about 60% of patients with NASH.
INCIDENCE OF MAJOR CARDIOVASCULAR AND CEREBRAL EVENTS IN PATIENTS WITH NAFLD AND IN CONTROLS OF GENERAL POPULATION DURING 10 YEARS OF FOLLOW UP: CORRELATION BETWEEN VASCULAR AND LIVER DAMAGE

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Introduction: NAFLD represents a risk factor for vascular damage. Carotid intima-media thickness (cIMT) is a known precursor of cardiovascular disease.

Aims: To evaluate 1) risk factors affecting the progression of cIMT and early carotid plaques (CP) in patients with NAFLD and in a control group from general population, 2) incidence of major cardiovascular and cerebral events in ten years of follow up 3) correlation between vascular and liver damage.

Material and Methods: 125 patients with NAFLD diagnosed by ultrasonography matched 1:2 for sex and age with subjects from general population underwent vascular evaluation in 2003 and were prospectively followed for a period of 10 years. In all subjects cIMT by ecocolor Doppler, clinical and biochemical data were evaluated at enrollment (time 0). After 10 years follow-up (time 1), 90/125 patients with NAFLD and 182/250 controls underwent abdominal ultrasonography to evaluate the presence of liver steatosis and a second cIMT measurements and CP evaluation, the remaining patients were lost at follow up. All clinical, biochemical data were recorded at time 0 and 1.

Results: At enrollment cIMT was significantly more elevated in NAFLD than in controls (0.87±0.23, vs 0.64±0.14, p=0.001) and the prevalence of CP significantly higher (21% v.s 6%, p=0.001).
After 10 years 54/182 (30%) controls developed steatosis. cIMT remained significantly more elevated in NAFLD than in controls who developed steatosis (0.95 ± 0.21 and 0.8 ± 0.13 mm, p= 0.004), while the higher prevalence of plaques was observed in controls who developed steatosis (50%, 46% and 60%, in NAFLD, controls without and with steatosis respectively). Thirty-five subjects developed major cardiovascular and cerebral events, the prevalence was significantly higher in NAFLD and in controls with steatosis (p=0.02). At logistic regression analysis variables significantly associated with events were age unit (O.R. 1.01, 95% C.I. 1.02-1.1, p=.004) systolic pressure (O.R. 1.05, 95% C.I. 1.01-1.08, p=.004), the presence of plaques (O.R. 3.78, 95% C.I. 1.5-8.8, p=.002.) and basal ALT >35 (O.R. 3.7, C.I. 1.08-14, p=.03).

Conclusions: In conclusion: subjects of general population are at high risk of developing steatosis throughout their life, major cardiovascular events have the same prevalence in NAFLD and controls with the higher prevalence in controls who developed steatosis and are related with liver damage. All subjects with steatosis have to be considered at high risk for cardiovascular complications.
SELECTIVE LXR ALPHA INTESTINAL ACTIVATION REDUCES HEPATIC INFLAMMATION AND FIBROSIS DURING THE DEVELOPMENT OF CHRONIC LIVER INJURY

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Introduction: Hepatic fibrosis represents the wound-healing response of the liver to chronic injury and it is characterized by increased and altered deposition of newly generated extracellular matrix. The pathogenesis of liver fibrosis is not fully understood, leading to a lack in effective therapies. Liver X receptors (LXR α/β) are important regulators of lipid metabolism. While in the liver LXRs regulate cholesterol and fatty acid metabolism, intestinal LXRα activation has been implicated in reverse cholesterol transport pathway leading to high levels of the anti-inflammatory HDL.

Aims: To evaluate the effect of the selective intestinal LXRα activation on the development of hepatic fibrosis associated to chronic liver injury.

Material and Methods: Male FVB/N mice (8-10 weeks old) with intestinal constitutive LXRα activation (iVP16LXRα) and their control (iVP16) were treated with twice weekly i.p. injection of Carbon Tetrachloride (1 µl/gr body weight) for 2 months.

Results: Immunohystochemistry for the macrophage marker F4-80 indicated lowered infiltration in iVP16LXRα liver (p<0.05). Gene expression of the pro-inflammatory cytokines IL-6, TNFα, and MCP-1, and of the transcription factor NF-Kb were reduced in iVP16LXRα CCL₄ mice (p<0.05, p<0.05, p<0.005 and p<0.005 respectively). Collagen synthesis and deposition was significantly reduced in iVP16LXRα mice compared to iVP16 as determined by type I collagen and TGFβ mRNA expression (both p<0.005) and by Sirius Red morphometry (p<0.0005). To study the mechanisms associated to the anti-inflammatory effect of intestinal LXRα activation, reverse cholesterol transport was proved by measuring intestinal gene expression of Abca1; indeed Abca1 expression was increased in iVP16LXRα mice (p<0.005) in line with the elevation of plasmatic HDL concentration in iVP16LXRα CCL₄ mice (p<0.005). Furthermore, intestinal LXRα activation is not associated to hepatic de novo lipogenesis as shown by gene expression of SREBP1c and FAS, and decreased triglyceride (p < 0.005) and cholesterol content (p<0,05).

Conclusions: Specific intestinal LXRα activation reduces liver injury by increasing the level of the anti-inflammatory HDL cholesterol, thus leading to decreased hepatic fibrosis. Selective intestinal activation of LXRα might be considered as a new therapeutic approach to reduce liver fibrosis avoiding the occurrence of hepatic steatosis as a side effects associated to systemic LXR induction.
BCL-3 REGULATES HEPATIC GLUCOSE AND LIPID METABOLISMS THROUGH INSULIN AND ASSOCIATED METABOLIC TRANSCRIPTION FACTORS

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Introduction: The NFkB-cofactor gene B cell leukemia-3 (Bcl-3) plays a critical role in altering the transcriptional capacity of the NFkB-subunits p50 and 52 and thus regulates inflammation. Bcl-3 was also identified as a transcriptional co-regulator of genes involved in cellular energy metabolism. However, its regulatory role in non-alcoholic fatty liver disease (NAFLD) has not been investigated yet.

Material and Methods: 8-12 week old male mice exhibiting a hepatocyte-specific overexpression of Bcl-3 (Bcl-3hepar) and wild type (wt) littermates were fed with a high-fat diet (HFD; 35.5% w/w crude fat (58kJ%)) plus drinking water enriched with fructose (55% w/v) and glucose (45% w/v) for 12 weeks to induce NAFLD. Gender- and age-matched mice received a control diet (CD; 5.4% w/w crude fat (13kJ%)) and normal drinking water. After the dietary period, biometric, serological, histological, FACS, Western Blot and qRT-PCR analyses were performed.

Results: HFD-feeding induced a pronounced metabolic derangement in Bcl-3hepar mice, which was characterized by enhanced hepatic cell death and steatosis from increased de novo lipogenesis (ACC, FAS, SREBP-1) and decreased beta-oxidation (CPT1, PPARalpha), hydrolysis (FXR, CES1) and release (MTTP) of fatty acids. In addition, Bcl-3hepar mice exhibited hyperinsulinaemia, but still a decreased expression of gluconeogenetic enzymes. PGC-1alpha, PPARalpha and PPARgamma as transcription factors which regulate hepatic glucose and lipid metabolism and also promote anti-inflammatory processes, were down-regulated in the liver of HFD-fed Bcl-3hepar mice as compared to the wt. Ex vivo-treatment of primary hepatocytes from Bcl-3hepar mice with oleic acid, fructose and glucose for 48h to induce intracellular lipid accumulation led to a diminished expression of PGC-1alpha, PPARalpha and PPARgamma, whereas these genes were not affected in wt hepatocytes. Moreover, metformin improves the metabolic and injurious phenotype of Bcl-3hepar mice in vivo by reducing hepatic steatosis via a decrease in lipogenesis (FAS) and elevated expression of PGC-1alpha, PPARalpha and PPARgamma. Metformin also caused a marked reduction of serum TNF-alpha, CCL2 levels and of intrahepatic leukocytes, in particular T cells, NK cells and macrophages.

Conclusions: In a high-fat dietary model of NAFLD, Bcl-3 is a hepatocellular factor that promotes metabolic dysfunction and insulin resistance by suppressing transcription factors of hepatic lipo- and gluconeogenesis.
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NUTRITIONAL WHEAT ALPHA-AMYLASE TRYPsin Inhibitors Synergize with a High Fat Diet to Worsen Non-Alcoholic Fatty Liver Disease

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Introduction: Non-alcoholic fatty liver is a broad disease spectrum ranging from mere steatosis to severe fatty liver inflammation (NASH) which may lead to cirrhosis and HCC. Hepatic injury via insulin resistance, hepatocyte lipoapoptosis, with subsequent activation of proinflammatory cells not only in the liver but also in visceral fat and the intestine appear to synergistically drive NAFLD progression to NASH. We recently identified wheat amylase trypsin inhibitors (ATIs) as nutritional activators of innate immunity via engaging the toll like receptor 4 (TLR4)-MD2-CD14 complex both in vitro and in vivo (Junker Y et al, J Exp Med 2012).

Aims: Our aim was to study the contribution of nutritional ATIs to the severity of experimental NAFLD/NASH.

Material and Methods: Male C57BI/6J mice received a carbohydrate and protein (casein) defined low or high fat diet (HFD, 13 KJ% vs 53KJ.% of calories as saturated fats), with or without 30% of the casein being replaced by gluten enriched in ATIs (0.3g/10g gluten) for 8 weeks. After 8 weeks blood, liver and peripheral adipose tissues were collected for biochemical and histological analysis. Histological sections were stained with hematoxylin and eosin and for hepatic lipid content (Sudan III). Inflammation related transcript levels were quantified by qPCR. F4/80 positive macrophages were quantified by IHC.

Results: Compared with the HFD controls, mice on HFD/ATI gained 11% more weight (36.9±0.8 vs. 33.2±0.5g), had a 6% increase in liver weight (1.43±0.06 vs 1.34±0.1g), significantly elevated serum triglycerides (TG) (p=0.0057) and hepatic lipid content (p=0.026), and increased serum ALT and AST levels.
The HOMA-IR was higher in the HFD/ATI than in the HFD group (>3 vs <2, resp.). Transcript levels of CD68 (macrophages), TNF-α and IL-1β were increased (trend and significantly, resp.), whereas alternative (and putatively anti-inflammatory) macrophage markers Arg1 and CD206 were significantly decreased (with a trend for PPAR-γ) in the HFD/ATI than in the HFD group. Histologically, F4/80+ macrophage numbers were elevated in the HFD/ATI fed animals. Moreover, mice in the HFD/ATI group showed a significant increase in mesenteric, inguinal and epididymal adipose tissue, and an upregulation of TNF-alpha and IL-1beta expression in epididymal, mesenteric and inguinal adipose tissue.

**Conclusions:** In summary, these data implicate nutritional ATIs from gluten containing cereals as important cofactors in the progression NAFLD and associated obesity, despite their caloric irrelevance.
IMPACT OF ROUTINELY LIVER BIOPSY DURING BARIATRIC SURGERY

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Introduction: Obesity is a worldwide epidemic and it is associated with serious co-morbidities. Metabolic syndrome is defined as a cluster of multiple cardiovascular risk factors including Non-alcoholic fatty liver disease (NAFLD). NAFLD is a wide spectrum of liver conditions, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), leading to fibrosis and potentially cirrhosis in 20 to 25% of patients with NASH. Different staged can only be diagnosed through liver biopsy (LB).

Aims: To assess liver biopsy results of patients undergoing RYGB

Material and Methods: Retrospective review of prospectively collected database of 265 consecutive patients who underwent BS. We analysed age, sex, initial BMI, liver function test, ultrasound and liver biopsies result.

Results: 51.2% were male, the average age was 44 ± 10.5 years. The BMI was 46.8 kg/m² ± 10.5. Only the 2.9% show a normal prequirurgic ultrasound. 49% had insulin resistance and 28% were diabetics. Mean ALT and AST were 1.5 – 1.2 upper the normal value respectively. We analysed NAS score and 9.81% had SE, 36.1 were borderline and 53.96% had NASH. When we assess fibrosis 16% did not have; 31.7% were F1 and 39.6% F2. We found cirrhosis in 13% of the LB.
During post op screening 2 patients had hepatocellular carcinoma and required liver transplantation. Non complication related LB.

Conclusions: NAFLD is clinically under recognised. Early detection of NAFLD by LB during BS can help to identify patients who will require screening for Hepatocarcinoma and portal hypertension. Our experience demonstrates that normal laboratory tests do not exclude the presence of advanced liver disease. The surgeon should be consider to perform routinely liver biopsy in all BS not only for diagnose and treatment also for legal concern in the future if liver failed.
MICROVESICLES AS NOVEL MARKERS IN NON-ALCOHOLIC FATTY LIVER DISEASE PROGRESSION

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver pathology and is the most complex to diagnose due to the lack of a clinically useful circulating biomarker. Microvesicles are a potential novel candidate. These submicron vesicles are shed from cells in both physiological and disease conditions, but their secretion profile in NAFLD progression have not been well documented.

Aims: We therefore investigated the effect of high-fat diet as a model of NAFLD on circulating and liver secreted microvesicles. Additionally we examined their expression of CD147, a proinflammatory molecule known to be secreted in microvesicles and linked to dysregulated tissue remodelling and hepatic fibrogenesis.

Material and Methods: Six week old male C57Bl/6 mice were fed a high-fat diet (45% kcal fat) or chow ad libidum for 12 or 50 weeks. At termination, blood and livers were harvested for circulating and secreted microvesicles respectively. These were isolated by ultracentrifugation of platelet-poor plasma or serum-free media collected from 18hr culture of liver sections. Microvesicle concentration and size were quantified using NanoSight while CD147 protein expression was determined by immunoblotting.

Results: Circulating microvesicle numbers were elevated in steatotic animals versus controls for both timepoints (by 2.2 and 12.6-fold, P<0.01). In contrast, we observed a significant decline in the corresponding liver secretome per gram tissue (by 2.5 and 25.9-fold, P<0.02). The microvesicle size distribution was unchanged. Immunoblotting revealed distinct changes in the liver microvesicle protein signature between the timepoints which also translated to CD147 expression; the high glycan form of the molecule was significantly reduced with high-fat feeding at 12 weeks (63% decrease, P<0.02) and was almost completely diminished by 50 weeks.

Conclusions: These results show dysregulation between circulating and tissue microvesicle numbers which was more prominent at 50 weeks, the more physiologically advanced stage of NAFLD. The proportional reduction in microvesicular CD147 secreted from the liver is an interesting phenomenon that could be explained by either the retention of the molecule within the liver during steatosis, or an alternate pathway for its translocation. Together these findings suggest that circulating microvesicles have potential utility as biomarkers of NAFLD.
HEPATIC ENCEPHALOPATHY AND GUT MICROBIOTA: IN VITRO MICROBIAL AND AMMONIA MODULATION BY PREBIOTIC, ANTIBIOTIC AND PROBIOTIC TREATMENT

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Introduction: Gut microbiome alteration represents a key factor in cirrhosis progression and onset of its neuropsychiatric complications. In particular, gut ammonia production by microbial activity is one of the main factors implicated in Hepatic Encephalopathy (HE) develop. Even if widely studied, the gut microbial dynamics during HE treatment with prebiotic, antibiotic and probiotic is only partially understood due to the different experimental approaches and inter-patient variability. Moreover, data on ammonia levels are usually related to circulating levels.

Aims: Using faeces from patients with cirrhosis, we investigated, how gut microbiota modulation by prebiotic, antibiotic and probiotic treatments effects microbial ammonia production using in vitro batch culture models.

Material and Methods: Fecal samples from six patients with cirrhosis (average age 66±3.3; Child-Pugh respectively A (n=5), and B (n=1); average MELD score 9±2.5) were used to inoculate independent 24-hour batch culture fermentations at controlled pH (6.8). Prebiotic (lactulose), antibiotic (rifaximin) and probiotic (VSL\#3) treatments were performed alone and in combination. Microbial populations were enumerated using culture independent Fluorescent in situ Hybridization (FISH), and ammonia concentrations were determined at 0, 4, 10 and 24 hours.

Results: Prebiotic and probiotic treatments modulated the cirrhotic microbiota including a significant increase in \textit{Bifidobacteria}, seen as beneficial microbiota components. Across the six patients, ammonia levels were reduced by prebiotic and antibiotic treatments with respect to control. The probiotic mixture VSL\#3, when considered alone, seems to increase ammonia levels during the 24 hours. The administration of VSL\#3 together with rifaximin and lactulose, decreased ammonia concentrations below the starting level.

Conclusions: This study emphasizes the potential of gut microbiota modulation as a target for relieving the symptoms of HE by regulating colonic ammonia production. Differences in ammonia production between antibiotic, prebiotic and probiotic treatments suggest a modulation in ammonia production rather than increased size of the “colonic ammonia sink” via microbial biomass alone, as a possible mode of action.
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METABOLIC PHENOTYPING OF BILE ACIDS - STANDARDIZED QUANTITATIVE ANALYSIS OF INDIVIDUAL BILE ACIDS WITH ONLY 10 MICROLITER SAMPLE – AN INTERNATIONAL INTER-LABORATORY RING TRIAL

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Introduction: The accurate quantitative determination of individual bile acids and their conjugates is very important in accessing liver damages as well as the microbiota regulation. We have developed and validated the very first Kit worldwide to standardize the analysis of individual bile acids from only 10 µL of either human plasma or human serum (16 bile acids) or mouse plasma samples (19 bile acids). Analysis of bile acids in faecal samples can also be performed with the kit.

Aims: The bile acid panel consists of cholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid, muricholic acids and their glycine as well as taurine conjugates. The Kit is based on the (U) HPLC-ESI MS/MS technology. An international ring trial with 14 participants has been successfully performed.

Material and Methods: A maximum of 85 samples with 10 µL each are processed on a specially designed 96-well filter plate within 2 hours. The analysis runtime is 5 min per sample. Compounds are detected using negative ESI MS/MS. 7-points calibration curves and 11 isotope labelled internal standards are used for quantitation. Pooled human and mouse plasma samples at different bile acid concentration levels have been sent to 14 ring trial participating laboratories for analysis.

Results: The assay has been rigorously validated according to the EMA guideline. LLOQ of 0.01-0.02 µmol/L is achieved for all target compounds. The individual calibration ranges cover both healthy and abnormal range. The international ring trial has shown excellent inter-laboratory comparability, accuracy (within 80% - 120%) and precision (CV < 30%) for all target bile acids.
**Conclusions:** This newly developed, simple and robust bile acids analysis Kit is proved to be a powerful tool in metabolic phenotyping and related biomarker research of metabolic, hepatic, pancreatic and cardiovascular disorders, in inflammatory diseases (e.g. sepsis) as well as in gastrointestinal cancer and microbiome regulation. The analysis of human and mouse plasma samples reveals that the bile acid profile of mice is quite different from that of human. While taurine conjugates of bile acids are prevalent and glycine conjugates are almost absent in mouse plasma, the situation is reversed in human samples. These findings are important for translational medicine. Excellent accuracy and precision in the ring trial has shown a huge potential for the standardization and harmonization in individual bile acids measurements using the Kit.

**Disclosure of Interest:** G. Krebiehl: Employee: Scientific Product Manager, Biocrates Life Sciences AG, H. Pham Tuan: : None Declared, D. Kirchberg: : None Declared, I. Zitturi: : None Declared, T. Koal: : None Declared
Clinical Efficacy of the Combination Therapy in the Treatment of Obesity

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Introduction: Obesity is one of the most common chronic diseases in the world. According to the modern principles, treatment of obesity should be complex and take into account many factors it causes.

Aims: Our aim was to evaluate the slimming effect of the combined therapy including S-adenosyl-L-methionine (AdoMet) and symbiotic (1.5 billion Alive bacteria *Lactobacillus rhamnosus* LGG with fruktooligosaharydamy) as an adjuvant of the dietary treatment of obese or overweight subjects.

Material and Methods: The study involved 37 patients with obesity. Among the patients were 22 women and 15 men aged 28-53 years (mean age: 44 ± 3.2 years). Body mass index (BMI) with 1 grade of obesity is found in 69% of patients, the average: 31.2 ± 2.1, grade 2 in 31% - an average of 36.4 ± 3.1. The content of leptin in serum of patients to treatment was 39.7 ± 2.5 ng/ml adiponektin (ADN) 4.2 ± 1.7 ng/ml of interleukin 6 (IL-6) - 15.2 ± 2.9 pg/ml. The presence of the small intestinal bacterial overgrowth (SIBO) was observed in 79%, and fatty liver in 93%. All patients were divided into two groups: the main group (MG) in addition to nutrition was appointed AdoMet 800 mg/day and 1.5 billion Alive bacteria *Lactobacillus rhamnosus* LGG with fruktooligosaharydamy for 3 months. Patients in the control group (CG) received only nutrition, behavioural therapy and adequate physical activity for 30 minutes/day.

Results: In patients with 1 degree of obesity found in CO BMI after treatment 27.1 ± 2.7, 2 degrees – 31.8 ± 2.9. Average rate of weight loss was 8.5 ± 1.16 kg compared with CG 4.5 ± 2.2 kg. Patients CO observed a statistically significant decrease in leptin 27.5 ± 2.1 ng/ml IL-6 to 2.7 ± 1.2 pg/ml, determined by reducing liver stiffness and ALT indicator (r<0.001 patients), ADN increased to 45.2% (p<0.01) patients improved indicators lipidohramm likely changes of cytokines and intestinal microflora in KG we not noted.

Conclusions: The inclusion of the AdoMet and 1.5 billion Alive bacteria *Lactobacillus rhamnosus* LGG with fruktooligosaharydamy to therapy in patients with obesity contributes to weight loss, normalization of intestinal microbiota and increases the effectiveness of treatment of these diseases. ADN role in the pathogenesis of obesity is confirmed by the fact that at lower weight, improved clinical status of patients after treatment, marked improve ADN.
MIR-21 INHIBITION AND FXR ACTIVATION SYNERGISTICALLY AMELIORATE DISEASE PATHOGENESIS IN A MOUSE MODEL OF NAFLD

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Introduction: Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver lesions, from simple steatosis to non-alcoholic steatohepatitis (NASH). Intrahepatic accumulation of fat represents a major triggering factor; still, disease pathogenesis remains incomplete. Recently, microRNAs (miRNA/miRs) have been linked to NAFLD. In particular, mir-21 may contribute to disease progression by targeting peroxisome proliferator-activated receptor α (PPARα). Indeed, PPARα and farnesoid X receptor (FXR) constitute promising NAFLD therapeutic targets.

Aims: We aimed to elucidate the role of miR-21 during NAFLD pathogenesis in mice, further evaluating the synergistic effect of miR-21 inhibition and FXR activation, using obeticholic acid (OCA).

Material and Methods: Wild type (WT; n=24) and miR-21 knockout (KO; n=24) mice were fed either a standard diet (SD; n=12) or a fast food diet (FF; n=12) for 25 weeks. 6 animals from each group had their diets supplemented with 60 mg/kg OCA (kindly provided by Intercept). Mice were weighed weekly while blood was collected and liver extracted and weighed at sacrifice. In parallel, human liver biopsies were obtained from morbid obese NAFLD patients at different disease stages (n=28). Liver samples were processed for histological analysis and determination of miR-21 and PPARα expressions by qRT-PCR and immunoblotting, respectively. Serum was used for biochemical parameters analysis.
**Results:** Our results show that, after 25 weeks, WT FF-fed mice develop NASH in parallel with an increase in both body weight and liver/body weight ratio, comparing with WT SD-fed mice ($p<0.05$). Further, they exhibited increased miR-21 ($p<0.05$) and decreased PPARα expressions ($p<0.05$). Strikingly, miR-21 and PPARα also displayed an inverse and significant correlation in human patients, increasing from steatosis to less and more-severe NASH ($p<0.05$). WT FF+OCA-fed animals displayed lower levels of miR-21, compared with WT FF-fed mice. KO FF-fed mice body weights and liver/body weight ratios were below WT FF-fed mice, as were serum levels of triglycerides and free fatty acids. Importantly, most of these beneficial effects were augmented in KO FF+OCA-fed mice ($p<0.05$).

**Conclusions:** In conclusion, our results indicate that miR-21 downregulation, likely leading to increased PPARα, together with FXR activation by OCA, ameliorate NAFLD pathogenesis, highlighting the therapeutic potential of novel synergistic therapies in NAFLD. (PTDC/BIM-MEC/0873/2012, SFRH/BD/88212/2012 and SFRH/BD/91119/2012, FCT, Portugal).
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MODULATION OF MUSCLE MICRORNA EXPRESSION PROFILES IN PATIENTS WITH NAFLD AND IN C2C12 CELLS INCUBATED WITH PALMITIC ACID

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Introduction: In the skeletal muscle, non-alcoholic fatty liver disease (NAFLD) associates with intramyocellular lipid deposition, mitochondrial dysfunction and insulin resistance (IR), particularly in obese patients. Further, recent evidences support a functional role for microRNAs (miRNA/miRs) in regulating muscle mitochondrial impairment and IR. Finally, tauroursodeoxycholic acid (TUDCA) is cytoprotective in both liver and muscle cells, in part, by stabilizing mitochondria.

Aims: Our aims were to profile global muscle miRNA expression profiles from patients at different NAFLD stages and validate their role, as well as of TUDCA, in insulin-resistant muscle cells.

Material and Methods: Muscle and matching liver biopsies were obtained from morbid obese NAFLD patients undergoing bariatric surgery. Muscle RNA was run in TaqMan MicroRNA arrays. qPCR array data was analysed using the HTqPCR package in Bioconductor. Differential expression analysis was performed with interquantile range values >1.5 using the lmfit function of the Limma package and the Benjamini-Hochberg conditional hypergeometric test algorithm. C2C12 cells were incubated with or without palmitic acid (PA), in the presence or absence of TUDCA, for characterization of the insulin signalling pathway, as well as mitochondrial and overall cellular toxicity.

Results: Our results show a progressive and significant increase in the expression of 6 muscle miRNAs from steatosis to more severe NASH (at least p<0.05).
This included miR-339-3p, which has been described to regulate glucose synthesis, and miR-361, found increased in type II diabetes (T2D) patients serum. Inversely, 8 miRNAs were decreased (at least p<0.05), including miR-20b, reported as down-regulated in T2D patients plasma. Incubation of C2C12 cells with PA inhibited the insulin-signalling pathway, while increasing mitochondrial dysfunction and apoptosis (at least p<0.05), all of which were prevented by co-incubation of cells with TUDCA (p<0.05). Of note, miR-339-3p was also increased by PA (p<0.05), targeting MAPK phosphatase-7 (MKP-7), a negative regulator of JNK, thus likely contributing for IR.

Conclusions: In conclusion, our results indicate that miRNAs associated with T2D and mitochondrial dysfunction are differently modulated with NAFLD severity in the muscle, with miR-339-3p arising as a novel mechanistic player. In addition, TUDCA attenuates muscle cells IR and lipoapoptosis and, as such, may ameliorate NAFLD-associated muscle dysfunction. (Supported by PTDC/BI-MEC/0873/2012, FCT, Portugal).
GUT-LIVER AXIS DERANGEMENT DUE TO LACK OF INFLAMMASOME ACTIVITY LEADS TO VISCERAL OBESITY AND NASH DEVELOPMENT

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Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common form of chronic liver disease and can lead to cirrhosis and hepatocellular carcinoma. Gut microflora alterations and bacterial translocation induced by specific dietary habits can elicit a proinflammatory and profibrogenic response. The NLRP3 inflammasome regulates intestinal homeostasis and mediates the release of IL1β and IL18 in response to cellular danger signals. Its role in NAFLD development is controversial.

Aims: Aim of the study was to investigate the role of NLRP3 inflammasome in a Western lifestyle diet model of NAFLD.

Material and Methods: Wild-type (WT) C57BL/6 and Nlrp3A350VneoR (Nlrp3−/−) mice were fed either a chow diet (controls) or a high-fat diet with fructose in drinking water (HFHC) for 12 weeks.

Results: Nlrp3−/− HFHC gained more weight (p<0.01), showed reduced energy expenditure and more fat mass (both measured by doubly labeled water, all p<0.05) with increased adipose tissue TNFα expression (p<0.05), and developed more hepatic steatosis measured by triglyceride content (p<0.01) compared to WT HFHC. HFHC increased intestinal permeability, as showed by reduced zonulin-1 expression in the caecum, that led to higher hepatic expression of TLR4 (p<0.01) and TLR9 (p<0.05) in Nlrp3−/− HFHC compared to WT HFHC. In the liver no differences were observed between Nlrp3−/− HFHC and WT HFHC in the expression of downstream SREBP-1c effectors of de novo lipogenesis (ACC, FAS and SCD-1) that were significantly increased in both groups.
On the other hand, compared to WT HFHC, \textit{Nlrp3}^{-/-} HFHC showed higher expression of the lipogenic transcription factor \textit{PPAR}\textgreek{y}2 (p<0.01) and of its downstream effectors \textit{FABP-4} (p<0.05) and \textit{CD36} (p<0.01) that regulate lipid uptake and storage. In addition \textit{Nlrp3}^{-/-} HFHC showed increased mitochondrial-oxidation of fatty acid (i.e., higher \textit{CPT1A} expression) that, associated to the reduced expression of the “master regulator” of the antioxidant response NRF2, led to increased superoxide production (measured by dihydroethidium staining) (all p<0.01). These series of events were associated to increased macrophage infiltration, type I collagen and MCP1 gene expression (p<0.05) in \textit{Nlrp3}^{-/-} HFHC mice only.

**Conclusions:** In the Western lifestyle diet, lack of NLRP3 inflammasome is associated with translocation of bacterial products that leads to severe metabolic alterations in both adipose tissue and the liver, and to NASH development.
THE URINE METABOLOMICS UNCOVERS KEY BIOMARKERS IN DIFFERENT STAGES OF NONALCOHOLIC FATTY LIVER DISEASE

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Aims: The urine with non-alcoholic fatty liver disease (NAFLD), including steatosis (normal liver function) and steatohepatitis (NASH) (abnormal liver function), was examined using metabolomics analysis in order to identify potential non-invasive biomarkers.

Material and Methods: Blood (separated serum for liver function or serum lipids assay) and urine sample were obtained from confirmed NAFLD without diabetic subjects with normal liver function (n=33) and NASH (with abnormal liver function) (n=45), and compared with healthy, age and sex-matched controls (n=30). The metabolic profile changes were analysed by liquid chromatography tandem mass spectrometry (LC/MS) with principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA). Furthermore, biochemical examinations were also carried out to compare healthy controls, NAFLD patients and NASH patients.

Results: Compared with the NAFLD patients, patients with NASH have abnormal liver function and high level serum lipids. Through urinary metabonomics, 31 metabolites are found between these two groups including 3-Methylxanthine, L-Histidine, 3-Indoleacetic Acid, Pyroglutamic acid, p-Hydroxyphenylacetic acid, N-Acetyl-DL-tryptophan, etc. These metabolites can be classified into nucleic acid and amino acid.

Conclusions: Statistical analysis identified a panel of biomarkers that could effectively separate NAFLD from NASH. These biomarkers can potentially be used to follow response to clinical diagnosis and therapeutic interventions.
Figure 1. A: Clinical information of human subjects with one-way ANOVA, significance was determined with $P$ value, compared with healthy group, *, $P<0.05$, **, $P<0.01$; compared with NAFLD group, #, $P<0.05$, ## $P<0.01$. (A, NASH group, B, NAFLD group, C, healthy group). B: S-plots following the PCA, PLS analysis between NASH group (A) and NAFLD group (B) (a, S-plots of PCA; b, S-plots of PLS; 1, ESI+; 2, ESI-)
A DISTINCT PROFILE OF LYSO-PHOSPHATIDYLCHOLINES AND AMINO ACIDS CHARACTERIZES NAFLD IN LEAN SUBJECTS

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is typically associated with obesity and the metabolic syndrome, however, approximately 10% of lean subjects (BMI < 25) also have NAFLD.

Aims: The aim of this study was to identify clinical and metabolic features of NAFLD in lean Caucasian subjects.

Material and Methods: Data from 247 patients allocated to one of 4 groups according to BMI and hepatic steatosis on ultrasound were obtained: lean healthy (BMI ≤ 25 kg/m², no steatosis, N=76), lean steatosis (BMI ≤ 25 kg/m², steatosis, N=57), obese healthy (BMI ≥ 30 kg/m², no steatosis, N=52), obese steatosis (BMI ≥ 30 kg/m², steatosis; N=62). A detailed clinical and laboratory examination including oral glucose tolerance test (oGTT) was performed. Metabolite profile was obtained by API 4000 triple quadrupole mass spectrometer (ABSciex) using the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences). Significant differences between groups were calculated using the false discovery rate (FDR) approach for metablomics analyses and ANOVA for comparison of clinical characteristics.

Results: Lean NAFLD subjects had fasting glucose concentrations and HOMA-IR similar to lean healthy subjects. However, lean NAFLD subjects had markedly impaired glucose tolerance as assessed by oGTT similar to obese NAFLD patients, and significantly different from lean healthy subjects (P<0.001). In the metabolomics analysis significantly lower levels of sphingomyelin (OH) C14:1, lysophosphatidylcholine (lysoPC) C18:0, lysoPC C17:0, phosphatidylcholine with diacyl residue sum (PC aa) C34:2 and higher levels of glutamic acid (FDR < 0.001 for all analytes) were found in lean NAFLD compared to lean healthy subjects.
A sum of lysoPC C18:0 and lysoPC C17:0 can separate lean healthy from lean NAFLD with a ROC area under the curve (AUC) of 0.76. Additionally, in lean NAFLD subjects higher levels of phosphatidylcholine with acyl-alkyl residue sum (PC ae) C 42:3, lysine and lower levels of alanine, tyrosine, valine and butyrylcarnitine (FDR < 0.001 for all analytes) were found when compared to obese NAFLD. ROC analysis of lysine, alanine and tyrosine discriminated lean and obese NAFLD subjects with an AUC of 0.88.

**Conclusions:** Although lean NAFLD patients have normal fasting glucose concentrations, the degree of glucose intolerance is similar to obese NAFLD patients. Furthermore, a distinct profile of lysoPCs and amino acids may be distinguishing indicators of the metabolic alterations linked to NAFLD in lean subjects.
INULIN LOWERS PLASMA SERUM AMYLOID A IN AN OBESE MICE MODEL

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Introduction: Obesity and related metabolic diseases such as type 2 diabetes, dyslipidemia and subsequently cardiovascular disease are known to be associated with alterations in intestinal microbiota. Chronic low-grade inflammation plays a key role in the pathogenesis of obesity and related metabolic disturbances.

Aims: In this study, we aimed to investigate whether dietary fiber-induced variations in gut microbial composition affect obesity-related adipose tissue inflammation and nonalcoholic steatoshepatitis (NASH).

Material and Methods: C57Bl/6 mice were fed a high fat-high cholesterol-high fructose diet (HFD) for 18 weeks in order to induce obesity-related adipose tissue inflammation and NASH. Modulation of the gut microbial composition was achieved by supplementing the HFD with the dietary fibers resistant starch (RS), inulin (IN) or guar gum (GG) (10% wt/wt). A glucose tolerance test was performed at 17 weeks. Various analyses on plasma and tissues were performed.

Results: GG, but not IN or RS, protected against diet-induced weight gain. Consequently, GG mice exhibited improved glucose tolerance. Interestingly, despite the similarity in bodyweight, IN mice showed a trend towards improved glucose tolerance. Expression studies showed that RS and IN did not affect the expression of inflammatory genes in mesenteric or gonadal white adipose tissue. However, without affecting hepatic triglyceride levels, IN significantly reduced plasma serum amyloid A (SAA), a marker of systemic inflammation predominantly produced by the liver.

Conclusions: Our preliminary data showed that dietary fibers have a differential impact on metabolic health. Whereas GG protects against diet-induced weight gain, RS does not seem to have beneficial effects on metabolic parameters. In contrast, independent of the obese state or hepatic triglyceride levels, IN significantly reduced plasma SAA which may indicate a reduction in hepatic inflammation. Our latest data on the impact of IN on hepatic inflammation and fat storage will be presented during the conference.
A WORSENING IN THE OGTT (ORAL GLUCOSE TOLERANCE TEST) AND HOMA (HOMEOSTATIC MODEL ASSESSMENT) INDEX AS A POSSIBLE PREDICTIVE OF LIVER STEATOSIS IN NON DIABETICS PATIENTS

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Introduction: Insulin resistance is one of the key factors in the pathophysiology of nonalcoholic fatty liver disease (NAFLD), involving a spectrum of conditions that include pure steatosis without inflammation, nonalcoholic steatohepatitis (NASH), fibrosis and cirrhosis.

Aims: The aim of the study was to verify the relation between insulin resistance, measured with OGTT and HOMA index, and the occurrence of liver steatosis.

Material and Methods: We included in the study 136 non diabetics outpatients (66 males; median age 56 years) followed at the Metabolism Outpatient Clinic from 2002-2012 with a history of dyslipidemia or hypertension, who underwent at least two OGTT, one at the baseline and one at least six months afterward (median interval of 52 months). In 67 patients we performed an abdominal US to verify the actual presence of liver steatosis; of 38 patients it was accessible a previous US, dated at least six years before.

Results: 70 patients (51.5%) had an abnormal baseline OGTT (IFG 25%, IGT 7.4%, IFG + IGT 18.4%, 0% diabetes). At the second OGTT 34.6% of them were still normal, while 9.6% had developed diabetes. Of the 67 patients, 52% had a normal US, while 48% had steatosis (13.4% mild, 7.5% moderate, 26.9% severe); of those 39 with a previous US, 44% showed a worsening in the severity of the steatosis. The outcome of the two OGTT was significantly associated with the severity of steatosis found both at the first and the second US, respectively (p=0.04 for both). However, a worsening of the OGTT did not predict the development of steatosis (p=0.63), even if there was a trend between the worsening of the OGTT and the worsening in the two US (p=0.06). According to the OGTT, the HOMA index was related with the severity of steatosis (p<0.01), but a difference in the HOMA was not related with a worsening in the US. At the multivariate analysis the HOMA index represents the only independent risk factor associated with the presence of steatosis (p=0.03).

Conclusions: While the study is retrospective and the sample size of the patients included in the study is limited, it is interesting to observe that an altered OGTT and the HOMA index may be considered an indirect signal of the presence of steatosis in non-diabetics patients. Moreover, the HOMA index may be a relevant parameter to evaluate the risk of liver steatosis.
THE ROLE OF N-3 FATTY ACIDS IN THE PATHOGENESIS OF RODENT NUTRITIONAL MODEL OF NON-ALCOHOLIC STEATOHEPATITIS

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Introduction: Non-alcoholic fatty liver disease (NAFLD) and subsequent non-alcoholic steatohepatitis (NASH) are probably the most common chronic liver diseases in western countries and have a high risk of development of liver cirrhosis associated with high morbidity and mortality.

Aims: The aim of the study was to determine effects of administration of n3 fatty acids (PUFA) in the methionine-choline deficient (MCD) dietary model of NASH and to assess the role of PUFAs in development and pathogenesis of NASH.

Material and Methods: For 6 weeks were male mice fed either with MCD or with chow. There were 4 groups of animals. Both experimental and control groups received from the beginning either PUFA or saline. Detailed liver histology and serum biochemistry were determined. Complete lipidomic (from liver and serum) analysis was performed by using ultra high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS). Principal Component Analysis (PCA) was used. Expressions BeadChip was used to profile and identify the differences in serum mRNA transcriptomes.

Results: Feeding with MCD (group M) resulted in histopathological changes of NASH and these changes were ameliorated in PUFA (MP). PUFA decreased cholesterol levels (P<0.001), ALT (P<0.01) and AST levels (P<0.01). MP developed less pro-inflammatory cytokine profile, had lower cytokine profile (P<0.01) than controls. Administration of PUFA led to lower serum concentrations of saturated and monounsaturated free FA and to higher serum concentrations of polyunsaturated FA in MP. Total serum lipid content and intensities of TAG was significantly lower in MP (P<0.001).
In the liver, diets without PUFA (control, M) had the highest intensity of arachidonic acid, diets with PUFA (CP, MP) contained higher amount of docosahexaenoic acid and eicosapentaenoic acid. MCD significantly increased amount of TAG in M and MP compared to C and CP (P<0.001). Higher content of TAG with longer carbon chains and more double bonds were found in MP compared to M. TAG and phospholipids were the most significant markers responsible for separation of the liver tissues according their diet using PCA.

**Conclusions:** We conclude that PUFA may play a causal role in the pathophysiology of NASH. In summary, PUFA have favourable effects on histopathological changes, serum markers of liver damage, FA compound. PUFA completely changed the metabolomic profile of the liver. We expect that PUFA may represent a promising way in prevention and treatment of NASH.
RELEASE OF CYTOKINES FROM SUBCUTANEOUS ADIPOSE TISSUE IN RESPONSE TO WEIGHT-REDUCING DIET IN OBESE WOMEN

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**Introduction:** Obesity-related metabolic complications are supposed to be associated with a pro-inflammatory state of adipose tissue characterized by an increased production and release of a number of cytokines in adipose tissue.

**Aim:** The aim of this study was to assess the effect of a 6 months’ weight-reducing hypocaloric diet on cytokine release from subcutaneous abdominal adipose tissue (SCAAT) into circulation.

**Material and Methods:** 9 obese women (BMI 34.1±1.4 kg/m\(^2\)) were submitted to a 6-months’ weight-reducing dietary intervention (DI) consisting of 3 months’ low calorie diet followed by a 3 months’ weight maintenance period. Before and at the end of DI, output of cytokines and proteins of acute phase from SCAAT was evaluated using Fick’s Principle. Thus, the levels of cytokines and proteins of acute phase (interleukin (IL)-6, IL-8, IL-1-receptor-antagonist (IL-1Ra), tumour necrosis factor-alpha, monocyte chemoattractant protein-1 (MCP-1), serum amyloid A and C-reactive protein) were measured in blood samples drawn from vein draining subcutaneous abdominal fat and from radial artery. Adipose tissue blood flow (ATBF) was assessed using the local Xe-clearance technique.

**Results:** The DI resulted in a decrease of body weight (96.5±3.0 vs 87.1±2.7 kg, p<0.01) associated with a decrease of insulin resistance as assessed by HOMA-IR (3.0±0.6 vs 1.4±0.2, p<0.05). DI did not alter ATBF (1.6±0.5 vs 1.3±0.2 ml/100g/min, NS). During the DI the net output of IL1Ra increased (p<0.01), while no change was detected in the outputs of IL-6 and MCP-1. No net output from SCAAT was found for other measured inflammation-related substances.

**Conclusions:** The results suggest that the beneficial metabolic effect of the weight-reducing DI might be associated with an increased release of the anti-inflammatory IL-1Ra from SCAAT while the releases of pro-inflammatory IL-6 and MCP-1 were not changed by DI.

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