**Background and Aims:** The prevalence of obesity has increased drastically in the last decades and this phenomenon constitutes today a serious health public problem. This obesogenic environment is characterized by an increase in adipose tissue mass. However, the adipocyte metabolism differs within (anatomical localization) and between individuals. The magnitude of adiposity is influenced by their potential growth (hypertrophy and/or hyperplasia) and their ability to triglyceride turnover. In fact, under a dysfunctional subcutaneous adipose tissue may result in the ectopic fat deposition in important metabolic organs such as visceral adipose tissue and liver. Progressively the hepatocyte loses the ability to metabolize the excess of lipids and can evoke to an inflammatory response and thereby progress to steatohepatitis (NASH). Our study aimed to assess adipose tissue metabolism and their metabolic changes during the development of NASH.

**Method:** Sixteen women and sixteen men with morbid obesity candidates to bariatric surgery were classified into the non-NASH group (n=8 per sex) and NASH group (n=8 per sex). Visceral and subcutaneous abdominal adipose tissue samples were immediately recollected before bariatric surgery. Histological analysis and immunoblot were performed to assess the adipose tissue dynamic and their impact on NASH progression according to sex.

**Results:** We found that in terms of hypertrophy only in Non-NASH group, the women had a significant increase in subcutaneous adipocyte size. However, the activity of adipocyte showed be heterogeneous according to the tissue and the sex. Of note, that in Non-NASH group the women showed an adipose tissue much more lipogenic in both tissues. By contrast, the adipose tissue of men showed a higher capacity to removal the triglycerides by enzymatic hydrolysis (lipolysis). Nevertheless, in the NASH group only we observed that the men visceral adipose tissue showed an increase of lipogenesis. Adipose tissue is a heterogeneous organ where the adipocytes have a sex-specific metabolism and thereby the response under the same pathological condition is different.

**SAT020**

**Hepatocyte-specific deletion of ERK5 worsens insulin resistance in a murine model of non-alcoholic fatty liver disease (NAFLD)**  
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**Background and Aims:** The extracellular signal-regulated kinase 5 (ERK5) is a member of the Mitogen-Activated Protein Kinases family highly expressed in hepatocytes, macrophages and stellate cells, and we recently generated hepatocyte-specific ERK5 knock-out mice (ERK5ΔHep). The aim of this study is to investigate the role of hepatocyte ERK5 in a murine model of NAFLD.

**Method:** ERK5ΔHep and control mice were fed with a high-fat diet (HFD) for 16 weeks. For glucose tolerance test (GTT) mice were injected with 1 g/kg BW glucose i.p. Insulin tolerance test (ITT) was performed by injecting 0.8 U/kg BW of regular insulin i.p. A murine hepatocyte cell line (MMH) was silenced using lentiviral vectors encoding shRNA for the ERK5 gene. Mitochondrial depolarization was assayed using the TMRE staining protocol. OXPHOS metabolism was measured by Seahorse.

**Results:** ERK5ΔHep mice exhibited impaired glucose tolerance and reduced insulin sensitivity in comparison to the control group. Body weight and food consumption were similar, while visceral fat was increased in ERK5ΔHep. MMH stably silenced for ERK5 showed reduced Akt activation following insulin stimulation. When cells were challenged with palmitic acid and then stimulated with insulin, Akt activation was completely abrogated in MMH/shERK5. In addition, measurement of mitochondrial membrane potential indicated a strong depolarization in MMH/shERK5 cells, which also showed an impairment of mitochondrial OXPHOS, indicating a profound impact of ERK5 deficiency on mitochondrial functions. In MMH/shERK5 cells, expression of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), a pivotal regulator of mitochondrial biogenesis and function, was up-regulated. Additionally, expression of TRIB3, a negative regulator of insulin signaling (through inhibition of Akt) under the control of PGC-1α was higher in MMHshERK5 cells, and its expression was further enhanced by palmitic acid. Increased expression of PGC-1α protein and TRIB3 was also observed also in liver tissues from HFD-fed ERK5ΔHep mice.

**Conclusion:** We have elucidated a novel pathway connecting expression of ERK5 in hepatocytes to the regulation of insulin sensitivity through PGC-1α, TRIB3, and Akt, which is relevant to the pathogenesis of NAFLD.

**SAT021**

MicroRNAs involved in the progression of non-alcoholic fatty liver  
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**Background and Aims:** The prevalence of obesity has increased drastically in the last decades and this phenomenon constitutes today a serious health public problem. This obesogenic environment is characterized by an increase in adipose tissue mass. However, the adipocyte metabolism differs within (anatomical localization) and between individuals. The magnitude of adiposity is influenced by their potential growth (hypertrophy and/or hyperplasia) and their ability to triglyceride turnover. In fact, under a dysfunctional subcutaneous adipose tissue may result in the ectopic fat deposition in important metabolic organs such as visceral adipose tissue and liver. Progressively the hepatocyte loses the ability to metabolize the excess of lipids and can evoke to an inflammatory response and thereby progress to steatohepatitis (NASH). Our study aimed to investigate the role of hepatocyte ERK5 in a murine model of NAFLD.

**Method:** ERK5ΔHep and control mice were fed with a high-fat diet (HFD) for 16 weeks. For glucose tolerance test (GTT) mice were injected with 1 g/kg BW glucose i.p. Insulin tolerance test (ITT) was performed by injecting 0.8 U/kg BW of regular insulin i.p. A murine hepatocyte cell line (MMH) was silenced using lentiviral vectors encoding shRNA for the ERK5 gene. Mitochondrial depolarization was assayed using the TMRE staining protocol. OXPHOS metabolism was measured by Seahorse.

**Results:** ERK5ΔHep mice exhibited impaired glucose tolerance and reduced insulin sensitivity in comparison to the control group. Body weight and food consumption were similar, while visceral fat was increased in ERK5ΔHep. MMH stably silenced for ERK5 showed reduced Akt activation following insulin stimulation. When cells were challenged with palmitic acid and then stimulated with insulin, Akt activation was completely abrogated in MMH/shERK5. In addition, measurement of mitochondrial membrane potential indicated a strong depolarization in MMH/shERK5 cells, which also showed an impairment of mitochondrial OXPHOS, indicating a profound impact of ERK5 deficiency on mitochondrial functions. In MMH/shERK5 cells, expression of peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α), a pivotal regulator of mitochondrial biogenesis and function, was up-regulated. Additionally, expression of TRIB3, a negative regulator of insulin signaling (through inhibition of Akt) under the control of PGC-1α was higher in MMHshERK5 cells, and its expression was further enhanced by palmitic acid. Increased expression of PGC-1α protein and TRIB3 was also observed also in liver tissues from HFD-fed ERK5ΔHep mice.

**Conclusion:** We have elucidated a novel pathway connecting expression of ERK5 in hepatocytes to the regulation of insulin sensitivity through PGC-1α, TRIB3, and Akt, which is relevant to the pathogenesis of NAFLD.
Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world; its prevalence has increased recently, accompanied by global obesity pandemic. It is a complex entity that arises from numerous genetic, environmental, behavioral and social factors. Non-alcoholic steatohepatitis (NASH) is part of the disease progression and is a preamble to more severe complications such as cirrhosis and hepatocellular carcinoma. Currently, the only tool to diagnose NASH is liver biopsy. The aim is to identify the different microRNAs (miRNAs) involved in NAFLD progression.

Method: 117 patients were recruited; liver biopsy and a blood sample were obtained; 30 were submitted to microarray assays and classified according to controlled attenuation parameter (CAP) and NAFLD activity score (NAS). Patients with CAP $\leq$ 232 dB/m, 0-point NAS and histopathological report without alterations, were the control group; with CAP $\geq$ 290 dB/m, NAS with 1 to 3 points and histopathological report with steatosis in more than 5% of hepatocytes, were the NAFLD group. Patients with CAP $\geq$ 290 dB/m, NAS $\geq$ 5 points and histopathological report with steatosis accompanied by inflammatory balloononing and fibrosis, were the NASH group. From blood samples, liver function tests, as well as fasting cholesterol, triglycerides, and glucose levels, were determined. RNA was extracted from liver tissue to analyze the miRNAs differential expression using the GeneChip miRNA 4.0 microarray; expression levels were compared with the Affymetrix TAC software using a fold change parameter $\geq$ 2 and $\leq$ -2, FDR $\leq$ 0.05 and $p \leq$ 0.001.

Results: Regarding the anthropometric characteristics, BMI had a statistical difference between control 27.8 kg/m², NAFLD 29.1 kg/m² and NASH 39.1 kg/m² $p \leq$ 0.0001; liver function profile, circulating lipids and fasting glucose did not change. The microarray analysis revealed a differential expression of 24 miRNAs in the NAFLD group, 23 were upregulated and 1 downregulated; miR-122-3p was expressed 9 times more than the control, followed by the miR-140-5p that showed almost 6 times more expression, the miR-200a-3p was 5 times more expressed, but also the miR-148b-3p and the miR-148a-5p were 4 times more expressed than the control; while the miR-6089-2 shows 2-fold drop expression compared to the control group. While NASH results disclosed 21 differentially expressed miRNAs compared to controls, miR-297, miR-3064-5p, miR3148 and miR-7844-5p showed more than 20 times decrease expression compared to the control group.

Conclusion: The results suggest that miRNAs differential expression could be used as potential NAFLD diagnostic and progression biomarkers; however, the mechanism by which these miRNAs are involved in the pathogenesis needs further investigation.

**SAT023**

Simultaneous intra-operative sampling from multiple anatomical sites reveals pro-inflammatory liver homing T cells in liver, adipose tissue and peripheral blood in patients with NASH

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is an inflammatory liver disease that can lead to fibrosis, cirrhosis and end stage liver disease. NASH has a multi-directional relationship with metabolic syndrome. Emerging evidence suggests an increase in peripheral blood T helper 1 (Th1) cells in NASH. However, the number and phenotype of T cells sampled simultaneously from adipose and liver tissue and peripheral blood in obese patients with NASH has not been studied to date. Here we test the hypothesis that a Th1 phenotype is dominant in all anatomical compartments.

Method: Patients undergoing bariatric surgery with NAFLD underwent simultaneous sampling of liver, visceral and subcutaneous adipose tissue and peripheral blood mononuclear cells for immunophenotyping by flow cytometry.

Results: We included 15 bariatric patients (median age 54); 6 with biopsy-proven NASH and 9 non-NASH. NASH patients had greater median BMI 49.8 (IQR 48.1–52.9) than non-NASH 42.0 (36.6–43.7), with mean ALT 91 vs 23 (p < 0.001) and median CAP score 374 dB/m vs 278 dB/m with a trend towards raised median liver elastography 11.2 Kpa (IQR 6.6–11.5) vs 7.7 Kpa (IQR 5.7–10.0). In peripheral blood, there was significantly greater CXCR3+ expression in CD4+ T cells in NASH (MFI 5447 vs 4377, p < 0.05) with a trend towards increased interferon gamma expression following stimulation with PMA. Overall expression of the liver homing marker CXCR6 was increased across all peripheral T cells in NASH by multiple linear regression analysis (p < 0.05). In particular, there were significantly more CD4+CXCR6+ T cells in NASH versus non-NASH. Among CD45+ cells extracted from liver tissue, patients with NASH had significantly more cytotoxic CD8+ T-cells (p < 0.01). In the visceral adipose compartment, Th2 cells (CD4+CRTH2+) sampled from obese non-NASH patients had significantly higher levels of CXCR6 expression compared to patients with NASH. Conversely there was a trend towards higher expression of CXCR6 on Th1 cells (CD4+CXCR3+) sampled from patients with NASH compared to those without (p = 0.059).

Conclusion: To our knowledge, this is the first study of immune cell phenotype in multiple anatomical compartments in this patient group. We find evidence of a pro-inflammatory, liver-homing phenotype in peripheral blood and adipose tissue and a dominant type 1 immune phenotype in all 3 compartments. Further investigation of the role of the adaptive immune system in the pathogenesis of NASH is warranted.