**Gastric Bypass Resolves Metabolic Dysfunction-associated Fatty Liver Disease (MAFLD) in low-BMI patients: A prospective cohort study**

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*Clinical and laboratory parameters*

Height and weight were measured, and BMI was calculated (weight in kg/height in m2). Insulin use and other medication were recorded during every follow-up visit. Blood was collected preoperatively and routine laboratory parameters including alanine-aminotransferase (ALT), glucose, glycated hemoglobin (HbA1c), fasting glucose, high- and low-density lipoprotein (HDL, LDL), triglycerides, serum free fatty acids (FFA), leptin and adiponectin were measured in the central laboratory of the University of Heidelberg Hospital. Insulin resistance was calculated according to the Homeostasis model assessment (HOMA-IR) formula 1. Patients were fasted for at least 6h before the operation as well as every follow-up visit. Insulin-injections were stopped for at least 12h before the scheduled follow-up visit or operation. Metformin was paused for 48h prior to the interventions. Therefore, the conditions were comparable for all measurements and biopsies.

*Histological assessment of liver biopsies*

Liver biopsies were formalin fixed and embedded in paraffin. Preparation and staining for hematoxylin&eosin (HE) as well as Sirius red was performed by the Institute of Pathology of the University Hospital Heidelberg in the routine set-up. Liver histology was assessed by expert liver pathologists (B. B. S. and P. S.) according to the established criteria by Kleiner et al. as well as according to Bedossa et al. 2, 3. The pathologists were blinded for the outcomes and the timepoints of the liver samples.

For the assessment of nitrotyrosin in the liver tissue, the paraffin blocks were sliced at 4µm using a microtom (Leica Rotary Microtome, Nussloch, Germany) and stained using hematoxylin and immunostaining. Target retrieval solution with a pH 9 was applied on the cut paraffin slides according to the manufacturer´s instruction (Dako, Hamburg, Germany). The primary anti-nitrotyrosin antibody (EM-30, Abcam, Berlin Germany) was applied according to the manufacturers` instruction. Staining was performed using the LASB-HRP-Kit (Dako, Hamburg, Germany) and counterstained with hematoxylin. Density of staining was assessed using a semi-quantitative scale (0-3 points) by two assessors blinded to the samples and outcomes (B.I.&K.S.). Five randomly selected areas per slide were assessed using a Zeiss Axiostar Plus microscope equipped with an Axiocam MRC camera (Zeiss, Jena, Germany). Results of the five selected areas were averaged.

*RNA-isolation and mRNA expression*

RNA was isolated from the liver tissue using the RNeasy Mini Kit (Qiagen, Hilden, Germany) from the liquid nitrogen stored samples according to the manufacturer’s instruction. RNA from the adipose tissue was isolated using the innuPREP RNA Kit (Analytik Jena, Jena, Germany) from the liquid nitrogen stored samples according to the manufacturer’s instruction. RNA-concentrations were measured using a NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, Germany). Gene expression was determined using real-time polymerase chain reaction (rt-PCR). Complimentary DNA (cDNA) was generated using the Maxima First Strand cDNA kit with dsDNAse according to the manufacturer´s instructions (Thermo Fisher Scientific, Germany). RT-PCR was performed with the KAPA SYBR Fast qPCR MasterMix according to the manufacturer´s instruction (NIPPON Genetics Europe, Düren, Germany) on a LightCylcer 480 system (Roche Life Science, Mannheim, Germany). The primer sequences were selected from the Harvard PrimerBank and custom made by ThermoFisher Scientific (Thermo Fisher Scientific, Germany). For normalization, 18s was used and fold-change was calculated using the the ΔΔCt (cycle threshold) method 4.

*Measurement of serum Fibroblast Grwoth Factor (FGF)-19, FGF-21, total bile acids, and serum nitrotyrosin*

Serum collected preoperatively and during the follow-up visits was stored at -80°C directly after the blood draw. Human FGF-19, FGF-21, total bile acids, and serum nitrotyrosin were analyzed in duplicates with enzyme-linked immunosorbent assays (ELISA) according to the manufacturer’s instruction and recommended dilutions (RayBiotech, Norcross, and BioVision, Milpitas, CA, USA). Optical density was measured according to the manufacturer’s recommendation at 450nm (Infinte 200 Pro, Tecan, Mainz, Germany) and data were analyzed using with the MagellanTM Data Analysis Software (Tecan, Mainz, Germany).

*Statistical Analysis*

Data are presented as mean±standard error of the mean (SEM) or as indicated. The Wilcoxon rank sum test was used as a paired test for not normally distributed data. A p-value <0.05 was considered significant. To investigate associations of improvement of liver histology (NAS) with preoperative BMI, weight loss and improvement of HbA1c, a Spearman correlation analysis was performed. GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis and presentation of the data.

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2. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; 41(6):1313-21.

3. Bedossa P, Poitou C, Veyrie N, et al. Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 2012; 56(5):1751-9.

4. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; 25(4):402-8.