**Supplementary Information**

**PREGNANCY AND LACTATION AFTER ROUX-EN-Y GASTRIC BYPASS EXACERBATE LIVER STEATOSIS IN OBESE RATS AND LEAD TO DIFFERENTIAL PROGRAMMING OF HEPATIC *DE NOVO* LIPOGENESIS IN OFFSPRING**

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*Serum biochemical analysis*

Before euthanasia, dams (F0) and offspring (F1) were food deprived for 8 h. Subsequently, the rats were euthanized by decapitation and total blood samples were collected, and serum was obtained through centrifugation at 1 200 g for 15 min. The serum was used for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG) and total cholesterol (CHOL) using the VITROSTM 4600 Chemistry Systemequipment(Ortho Clinical DiagnosticsTM, USA).

*Lipid profiles of the F0 and F1 groups*

Fragments of the left lobe of the livers from the F0 and F1 were collected and weighed before submitting to an overnight total lipid extraction protocol using chloroform-methanol solution (2:1, v/v).1 After filtration and evaporation, the vials were weighed to determine total fat content (final weight minus initial weight). Subsequently isopropanol was added to the samples and TG and total CHOL liver content were measured with commercial colorimetric kits, according to manufacturer's instructions (Laborclin®, Bioliquid, Pinhais, PR, BR).

*Liver histopathology in the F0 and F1 groups*

Another liver fragment was removed from the left lobe of each of the F0 and F1 rats, and was fixed in 4% paraformaldehyde for 24 h, before dehydrating in alcohol, diaphanizing in xylol and embedding in Paraplast® (Sigma-Aldrich, Saint Louis, MO, USA). Slices of 3 μm in thickness were stained with hematoxylin and eosin. For steatosis analyses, 3 sections from each liver were analyzed by two blind researchers, using a light microscope (Olympus DP71; Tokyo, Japan). The liver histopathology was examined and graded according to the magnitude of steatosis, based on Brunt’s classification as: grade 0, <5% of hepatocytes with lipid vaccuoles; grade 1, >5% to 30% of hepatocytes with lipid vaccuoles; grade 2, >30% to 60% with lipid vaccuoles; and grade 3, > 60% of hepatocytes with lipid vacuoles.2Other histopathological features as: presence of apoptosis, necrosis, inflammatory infiltrate, hyperemia and fibrosis were analyzed by a trained pathologist in accordance with the pathological criterions described by Liang et al.3

*Quantitative mRNA expressions in the livers of the F0 and F1 groups*

Another liver fragment from each of the rats of the F0 and F1 groups was stored in 150 µL of RNAlater® solution (Life Technologies, Carlsbad, CA, USA). The liver RNA was isolated using a commercial kit (Promega, Madison, USA) and the reverse transcription of mRNA was performed using the Superscript II kit (Invitrogen, Carlsbad, CA, USA). The transcripts were detected using the 7500 Real-Time PCR system with SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). The Primer sequences used were designed and purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA) and are shown in Supplementary Tab. S1. The expression of each gene was normalized to that of the internal control, glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), that is stable/unchanged across bariatric surgery animal models.4 Absolute gene expression was calculated using standard curves (108-103 copies/DNA molecules in 2 μL), produced from the gene amplification products separated on 2% agarose gels.

*Protein expression in the livers of the F0 and F1 groups*

A fragment of the liver from each of the rats of the F0 and F1 groups was collected and placed in a microtube containing protein extraction buffer (100 mM tris pH 7.5, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride and 1% Triton-X 100). Proteins were extracted using a Polytron MA 102 generator (model MA 102/Mini; Piracicaba, SP, BRA) and protein concentration was measured by the Bradford dye method. For SDS gel electrophoresis and Western blot analysis, 80 µg of samples were homogenized with loading buffer containing beta-mercaptoethanol. The proteins were separated by electrophoresis and afterwards transferred to nitrocellulose membranes before incubating with primary antibodies to acetyl-CoA carboxylase (ACC), phospho (p)-ACC, fatty acid synthase (FASN), stearoyl-CoA desaturase-1 (SCD-1), carnitine palmitoyl-transferase-1α (CPT-1α), microsomal triglyceride transfer protein (MTTP), p-mammalian target of rapamycin (pmTOR), or mTOR; α-tubulin was used as an internal control, that is stable/unchanged across bariatric surgery animal models.4-8 The antibodies used are shown in Supplementary Tab. S2. Visualization of protein bands was performed by incubating the membranes with specific secondary antibody and with chemiluminescent reagents, followed by registration of bands using the L-Pix Chemi Express image capture system (Loccus Biotechnology®, Cotia, SP, BRA). Band intensities were quantified by optical densitometry using the LabImage 1D software of image analysis (Loccus Biotechnology®, Cotia, SP, BRA).

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