SUPPLEMENTAL DATA

SUPPLEMENTAL MATERIALS AND METHODS

Energy contents of the diets

The HF diet provides 24.6 kJ/g Gross energy (60 % as fat, 20% as carbohydrate and 20% as protein). The chow diet provides 17.3 kJ/g Gross energy (18 % as fat, 58% as carbohydrate and 24% as protein). Perioperatively, rats were fed liquid Ensure in order to prepare the digestive tract for surgery and to reduce gut distension. The Ensure diet provides 4.64 kJ/g Gross energy (24 % as fat, 61% as carbohydrate and 15% as protein).

Surgery

All rats were operated under isoflurane anaesthesia. All gut anastomoses were performed an end-to-side technique with 7.0 PDS II suture (Ethicon Inc., Sommerville, NJ, USA). The duodenum was then ligated with 1.0 silk. The gastric suture was performed in two layers using 5.0 vicryl (Ethicon). All anastomoses were executed by a hand-sewn Connell technique. The abdominal wall was closed in two layers using a 5.0 vicryl (Ethicon).

Pre- and postoperative care

Preoperatively, all rats were given a liquid diet (vanilla flavoured Ensure, Abbott Nutrition, St Laurent, QC, Canada) for 48 hours and then fasted for 18-20 hours before their scheduled surgery. Wire mesh support grids were placed in rats’ cages to ensure that they not eat any sawdust. An antibiotic, Ceftriaxone (75 mg/kg i.m.) as well as an analgesic, Buprenorphine (0.02 mg/kg s.c.) were administered before surgery.
Post-operatively, rats were given water ad libitum. Parenteral rehydration solutions (sterile isotonic saline and dextrose 5%) and analgesics Buprenorphine and Ketoprofen (5mg/kg s.c.) were s.c. administered for the first 3 days post-surgery and continued if needed. Starting on the day 3, rats were offered increasing amounts of liquid diet (Ensure). Gradual transition to the same solid diet as fed pre-operatively (HF or chow, respectively) was provided during the first post-operative week. The rats resumed to ad libitum solid diet on day 7 post-surgery.

**Plasma hormones and metabolites**

In order to inhibit activity of proteinases, part of the collected blood was mixed with aprotinin (500U/ml blood) and DPP IV inhibitor (10µl/ml blood) before centrifugation (3500 rpm for 10 min at 4°C). In plasma samples destined to acylated ghrelin determination, HCl 1N (100µl/ml plasma) was added before a second centrifugation (3500 rpm for 5 min at 4°C). Supernatants were then transferred in separate tubes and kept frozen at -80°C until later GLP-17-36, PYY, ghrelin and GIP measurements. Separate blood samples, without being treated with proteinases inhibitors, were centrifuged (3500 rpm for 10 min at 4°C) and collected plasma was stored at -20 °C until later determination of glucose, insulin, peptide C, leptin, triglycerides, NEFA and cholesterol levels.

Commercially available assay kits were used for the measurement of plasma insulin (ultrasensitive ELISA, Alpco Diagnostics, Salem, NH, USA), C-peptide (ELISA, Alpco), leptin (ELISA, Alpco), glucose (enzymatic assay, Wako Diagnostics, Richmond, VA, USA), TG (enzymatic assay, Randox Laboratories, Crumlin, UK), NEFA (enzymatic assay, Wako Diagnostics), Cholesterol (enzymatic assay, Randox Laboratories), GLP-17-36 (ELISA, Alpco), PYY (RIA, Millipore, St Charles, MO, USA), GIP (ELISA, Millipore) and acylated ghrelin (EIA, SPIBio-Bertin Pharma, Montigny le Bretonneux, France).

**Brown adipose tissue gene expression**

Total RNA was isolated from 100 mg of interscapular BAT using QIAzol lysis reagent (Qiagen, Mississauga, ON, Canada) and further purified by using the RNeasy Lipid Tissue Mini kit (Qiagen). RNA
concentrations were estimated from absorbance at 260 nm using a Multiskan Spectrum (Thermo
Scientific, Milford, MA, USA). The purity of the DNA template was estimated from the ratio of
absorbance 260nm/280nm of 1.8-2.0. The cDNA was synthetized using the expand reverse transcriptase
50 U/µl (Roche Diagnostic, Laval, QC, Canada) and it was diluted 1:25 in DNase-free water before
further using. The SYBR Green I dye (SYBR® Green JumpStart™ Taq ReadyMix, Sigma Aldrich,
Oakville, ON, Canada) was used for detection of PCR products. The primers sequences were forward 5’-
GACATCATCACCTTCCCGCT-3’ and reverse 5’-GTCATCAAGCCAGCCGAGAT-3’ for UCP1,
forward 5’-CAGAAGGCCCTGAAGGAGAA-3’ and reverse 5’-TAGTCCCACCTTGGCAGGAGAA-3’ for
COX4, forward 5’-CGGAAGCACCACCGCGAGTA-3’ and reverse 5’-
GCAGCTTCAGGTTTGTCCGAATA-3’ for CPT1b, and forward 5’-
TCCTGTTACTATTATGAATCAAGCC-3’ and reverse 5’-AAACCATAGCTGTCCATCATCC-3’
for PGC1a.

The real-time quantitative PCRs were performed using a 7900 HT fast real time PCR system (Applied
Biosystems, Foster City, CA, USA). At the end of the runs, melting curve analysis was performed.
Representative samples of each group were run on agarose gel and sequenced to ensure the specificity of
the amplification. Standard curves were used for relative quantification. All samples were run in
duplicates and the mean values were used for further analysis.
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Schematic representation of SG, DS and BPD/DS procedures in rat. SG was performed by excising the aglandular forestomach (estimated to be 60% of the gastric capacity in the rat) and suturing the remaining gastric lumen along with the smaller gastric curbure. To create the DS, gut was transected 50-cm above the ileocecal junction, and the proximal part of the cut intestine was anastomosed to the ileum at about 20 cm from the ileocecal junction. Thus, a 20-cm common limb was created. The distal part of the cut intestine was anastomosed to the duodenum and resulted in a 30-cm alimentary limb. The duodenum was then ligated between the duodenal anastomosis and biliopancreatic ducts. Thus, the biliopancreatic limb was isolated from the food transit. The nutriments (conducted by the alimentary limb) join the biliopancreatic secretions (channelled by the biliopancreatic limb) while transit the common limb. Complete BPD/DS model includes both SG and DS procedures.

Supplemental Figure 2. Effects of sham surgery on body weight. Sham procedure involving sham gastrectomy with suture and intestinal anastomoses (Sham anastomoses) compared with simplified sham procedure that involves the handling of intestines and stomach (Sham handling).

Supplemental Figure 3. HOMA-IR index prior (A) and at 8 weeks after the surgery (B) in preoperatively in BPD, DS, SG and sham-operated rats. * P<0.05 vs sham HF; † P<0.05 for BPD/DS vs sham HF PW.

Supplemental Figure 4. Cumulative body weight (A) and daily energy intake (B) in BPD, DS, SG and sham-operated animals for all experimental period. * P<0.05 vs. sham HF; † P<0.05 for BPD vs. sham HF PW.
BPD/DS

biliopancreatic limb

alimentary limb

common limb

sleeve gastrectomy