SUPPLEMENTAL MATERIALS AND METHODS

Subjects and tissue sampling

The main study sample included severely obese subjects (BMI $\geq 40$ kg/m$^2$), without T2D, undergoing biliopancreatic diversion at the Quebec Heart and Lung Institute, Laval University (Quebec City, Canada). Insulin and glucose levels were assessed in the plasma of each subject to calculate the homeostatic model assessment of insulin resistance (HOMA-IR). Twenty of these subjects (10 women and 10 men) were assigned to two groups matched for age ($\pm 10$ years), gender and body mass index (BMI) ($\pm 5$ units), but with either relatively low (<3) or high (>7) IR according to their HOMA-IR. None of these subjects received a therapy with estrogen, hypoglycaemic agents and cholesterol- or TG-lowering agents. Duodenum specimens were obtained during the surgery. The intestinal samples were immediately transferred to the laboratory for functional studies (e.g. de novo lipogenesis, apo B-48 biogenesis and chylomicrons assembly and secretion). The remaining tissue was immediately frozen in liquid nitrogen and stored at –80 °C for subsequent analyses. Written informed consent was obtained from all subjects. The project was approved by the ethics committees of Quebec Heart and Lung Institute, Laval University (Quebec City, Canada) and Sainte-Justine Research Center (Montreal, Canada).

Anthropometric, lipid profile and glucose homeostasis

BMI was measured on the morning of the surgery. Similarly, overnight fasting blood samples were drawn on the morning of the surgery. Total cholesterol, free cholesterol and triglyceride levels were estimated in the plasma by colorimetric enzymatic kits (Roche Diagnostic, Indianapolis, USA). Glucose was assessed using the glucose oxidase method and insulin was quantified with the ultrasensitive insulin assay on the Access® immunoassay system (Beckman Coulter, Brea, USA). The HOMA-IR index was calculated using the following formula:

$$\text{HOMA-IR} = \frac{\text{fasting insulin (µU/mL)} \times \text{fasting glucose (mmol/L)}}{22.5}.1$$

Protein expression analysis

Intestinal samples were homogenized with a polytron in the lysis buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na$_2$HPO$_4$, 1.47 mM KH$_2$PO$_4$ (pH 7.4), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, 2 mg/ml pepstatine). Homogenates were agitated at 4°C for 1h in presence of 1% Triton and then centrifuged at 13,000 rpm for 10 min. The Bradford assay (Bio-Rad Laboratories, Mississauga, Canada) was used to determine protein concentration. Homogenates were prepared for Western blotting as described previously.2 Same protein amounts (30 ug) were loaded on the SDS-PAGE and the β-actin protein was used as an internal control. The following antibodies (directed against human) and dilutions (1:1000 unless otherwise specified) were employed: mouse anti-β-actin (1:40000, Sigma Aldrich, St. Louis, USA); anti-Akt
(#ab32902) and anti-TNF-α (#ab66579) from Abcam (Cambridge, USA); anti-intestinal-fatty acid binding protein (I-FABP) and anti-liver-fatty acid binding protein (L-FABP) antibodies were raised in rabbits after injection of recombinant proteins; anti-microsomal transfer protein (MTP) was kindly provided by John Wetterau and Harris Jamil (Bristol-Myers Squibb Research Institute, USA); anti-SAR-1B (1:2000) was kindly provided by Randy Schekman (University of California, USA); anti-PCSK9 was kindly provided by Geneviève Dubuc and Jean Davignon (Clinical Research Institute of Montreal, Canada); anti-nuclear factor kappa B (NF-kB) p65 subunit (sc-372G) and anti-I-KappaB-alpha (IKB-α) (sc-1643) were obtained from Santa-Cruz Biotechnology (Santa-Cruz, USA); anti-phospho-Akt Ser473 (#9271), anti-phospho-AMPKα Thr172 (#40H9), anti-AMPKα (#2532), anti-phospho-p38 MAPK Thr180/Tyr182 (#4631), anti-p38 MAPK (#9212), anti-phospho-JNK Thr183/Tyr185 (#9251), anti-JNK (#9252), anti-phospho-Acetyl-CoA Carboxylase (ACC) Ser79 (#3661) and anti-ACC (#3662) were obtained from Cell Signaling Technology (Boston, USA).

**Messenger RNA expression**

Total RNA was isolated from intestinal tissue using the RNeasy lipid tissue extraction kit and on-column DNase treatment following the manufacturer’s recommendations (Qiagen, Valencia, USA). RNA quality was assessed using the Agilent Technologies 2100 bioanalyzer (Agilent, Santa Clara, USA). Complementary DNA was generated using the Superscript first strand synthesis system (Invitrogen, Carlsbad, USA). Real-time cDNA amplification was performed in duplicate using SYBR Green with the 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA) and was carried out for 40 cycles. Target genes amplifications were normalized using expression levels of ATP synthase 5 subunit O (ATP5O). The relative mRNA fold changes between groups were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences used for each gene are shown in the following Table. Validation was performed to ensure optimal amplification efficiency of the target and reference gene cDNA.

**Table 1: Primer sequences used for quantitative RT-PCR**

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Sense primers</th>
<th>Anti-sense primers</th>
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<tbody>
<tr>
<td>ABCA1</td>
<td>TGCTGTGGAAGAACCTCACTT</td>
<td>CGAACAGAGATCAGGATCAGG</td>
</tr>
<tr>
<td>CETP</td>
<td>CTCTGCCATTGACCTCCAGA</td>
<td>AGGTAGCAGTCAGGGGCATC</td>
</tr>
<tr>
<td>TNFA</td>
<td>CCCCAGGGACCTCTCTCTAA</td>
<td>TGGGCTACAGGCTTGTCACT</td>
</tr>
<tr>
<td>IL1B</td>
<td>AGCCATGGCAAGATGACTCT</td>
<td>GGCCATAGCGCTTCAAGAAC</td>
</tr>
<tr>
<td>TLR4</td>
<td>AGAACTGCAGGTGCTGGATT</td>
<td>ATGCCCATCTTTCAATTGTC</td>
</tr>
<tr>
<td>ATP5O</td>
<td>GCGATGCTTCCATCACCTCTG</td>
<td>TGGCATAGCGACCTTCAATA</td>
</tr>
</tbody>
</table>
Lipid peroxidation appraisal

The amount of malondialdehyde (MDA) in tissue homogenates was determined by HPLC as described previously. Briefly, proteins were precipitated with sodium tungstate (Na₂WO₄) and the supernatant was incubated with thiobarbituric acid (TBA) at 90 °C for 60 min. The TBA₂-MDA chromogen was extracted with 1-butanol, dried under nitrogen, resuspended in KH₂PO₄/methanol (70:30, pH 7.0) mobile phase before detection by HPLC coupled to a fluorescent detector. Conjugated dienes were extracted from tissue homogenate using heptane/isopropyl alcohol (2:1) and measured spectrophotometrically in heptane layer. Results were normalized for total protein concentration.

Antioxidant enzymes

Activities of the antioxidant enzymes were measured in tissue homogenates using adapted protocols from Pippenger et al. For catalase activity, tissue homogenates (10 ug) were mixed with phosphate-buffered saline (PBS) solution, pH 7.0, 10 mM hydrogen peroxide (H₂O₂) and absorbance was monitored by spectrophotometry at 240 nm for 3 min. For glutathione peroxidase (GPx) activity, tissue homogenates (150 ug) were incubated with PBS solution, pH 7.0, containing 1 mM glutathione (Sigma Aldrich, St. Louis, USA), 0.6 units of glutathione reductase (Sigma Aldrich, St. Louis, USA), and 100 mM β-nicotinamide adenine dinucleotide 2'-phosphate-reduced (NADPH) tetrasodium salt (Sigma Aldrich, St. Louis, USA) and 1.5% H₂O₂. Absorbance was monitored at 340 nm for 5 min. To assess superoxide dismutase (SOD) activity, tissue homogenate (130 ug) were incubated in presence of superoxide radicals (O₂⁻), which were generated by the addition of xanthine and xanthine oxidase (Sigma Aldrich, St. Louis, USA), and oxidation was followed by spectrophotometry at 550 nm for 5 min. All experiments were normalized for total protein concentration.

Inflammation markers

The levels of intercellular adhesion molecule-1 (ICAM-1) and Interleukin-6 (IL-6) were determined using ELISA kits from Abcam (Cambridge, USA) and from RayBiotech (Norcross, USA), respectively. Assays were performed according to the manufacturer's protocol. Results were normalized for total protein concentration.

Intestinal organ culture

Fresh intestinal specimens were cleared of mesentery, split longitudinally, washed in culture medium, and cut into explants (3 × 7 mm). Explants were randomly transferred onto lens paper, with the mucosal side facing up, in organ culture dish (Falcon Plastics, Los Angeles, USA). Explants were cultured in serum-free Leibovitz L-15 medium with anti-proteases and antibiotics according to the technique described previously. To determine the responsiveness of fresh intestinal explants to oxidative stress, they were exposed to iron (0.2 mM)-
ascorbate (2 mM) (1:10) system-mediated lipid peroxidation. At the end of a 6 h culture period, the degree of lipid peroxidation was evaluated by measuring conjugated dienes in tissue homogenates. Similarly, fresh intestinal explants were incubated with lipopolysaccharides (LPS, 100 µg/ml, Sigma Aldrich, St. Louis, USA) for 6 h to examine the inflammatory response by assessing IL-6 secretion. All experiments were normalized for total protein concentration. At the end of explant incubation, integrity of the intestinal mucosa was examined (n=10/group) by assessing various variables related to cell proliferation ([³H]-thymidine incorporation), differentiation (sucrase), function (lactase, maltase), glucose metabolism (uptake and oxidation of glucose), cell viability and membrane integrity (leakage of lactate dehydrogenase and alkaline phosphatase to the medium) as assessed previously.

Table 2: Integrity assessment of intestinal explants in culture.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Insulin-sensitive obeses</th>
<th>Insulin-resistant obeses</th>
<th>P values</th>
</tr>
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<tbody>
<tr>
<td>Lactase (µmol/min/g protein)</td>
<td>2.4 ± 0.4</td>
<td>2.6 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sucrase (µmol/min/g protein)</td>
<td>41.2 ± 5.5</td>
<td>39.1 ± 6.2</td>
<td>NS</td>
</tr>
<tr>
<td>Maltase (µmol/min/g protein)</td>
<td>99.0 ± 11.6</td>
<td>113.4 ± 14.8</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>213.9 ± 17.3</td>
<td>241.4 ± 19.8</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphatase (µmol/min/g protein)</td>
<td>39.9 ± 4.1</td>
<td>36.2 ± 5.6</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose uptake (% of total radioactivity)</td>
<td>27.8 ± 5.4</td>
<td>25.7 ± 6.3</td>
<td>NS</td>
</tr>
<tr>
<td>CO₂ production (% of total radioactivity)</td>
<td>3.8 ± 0.6</td>
<td>3.4 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>[³H]-thymidine incorporation (dpm/µg DNA)</td>
<td>295 ± 18</td>
<td>286 ± 22</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD (n=10) and t-test p values are indicated. NS: Not significant.

**De novo lipogenesis assay**

Intestinal explants were incubated in the presence of 1 µCi of [1-¹⁴C]-acetic acid (Perkin Elmer, Waltham, USA) for 3 h. After incubation, explants were homogenized in PBS containing 1% (vol/vol) Triton X-100. Total lipids were extracted with 2:1 (vol/vol) chloroform-methanol. Solvents were dried under nitrogen gas, and resuspended in chloroform to assess incorporation of [1-¹⁴C]-acetic acid into lipid by liquid scintillation counting.

**Apo B-48 and triglyceride-rich lipoproteins synthesis**

After a 30-min stabilization period, intestinal explants were incubated in presence of a micellar mixture (6.6 mmol/l sodium taurocholate, 1 mmol/l oleic acid, 0.5 mmol/l monoolein, 0.1 mmol/l cholesterol, and 0.6 mmol/l phosphatidylcholine) containing 0.5 µCi of [1-¹⁴C]-oleic acid (specific activity: 53.9 mCi/mmol) or 300
μCi $[^{35}\text{S}]$methionine (specific activity: >1000Ci/mmol) purchased from Perkin Elmer (Waltham, USA). Intestinal explants were cultured for 3 h at 37°C in an atmosphere of 5% CO$_2$. Determination of secreted triglyceride-rich lipoproteins (TRL) was performed by spinning the medium at 100,000g for 2.26 h at a density of 1.006 g/ml as described previously. Radioactivity in each fraction was measured by liquid scintillation counting. For determination of apo B-48 synthesis and secretion, the medium was incubated with excess anti-apo B polyclonal antibodies and protein A/G for 18 h at 4°C. The immunoprecipitates were separated using a linear 4–20% acrylamide gradient gel and counted by liquid scintillation as described previously.

**Statistical analyses**

Differences in parameters between groups were tested using paired t-test and repeated measures analysis of variances. Relative mRNA expression values ($\Delta\Delta$Ct) between groups were compared using the Wilcoxon signed-rank test. Differences were considered to be statistically significant at $p<0.05$. Statistical analyses were performed with JMP software (SAS Institute, Cary, USA).
REFERENCES


