Supplementary Material

Materials and Methods:

Quantitative PCR (qPCR):

Intestinal tissue was homogenized using the TissueLyser (Qiagen, Stanford, CA, USA). Total RNA was isolated using the Qiagen RNeasy mini-kit (Qiagen, Stanford, CA, USA) and DNase treated using DNase1 (Qiagen, Stanford, CA, USA). Genes tested: GATA4, Ileal lipid binding protein (ILBP), Cyp7A1, Cyp8B1, Cyp27A1, ApoA1, SR-BI, Bile salt export pump (BSEP), Na/Taurocholate cotransporting polypeptide (NTCP), Farnesoid X receptor (FXR), Short heterodimer protein-1 (SHP-1), Liver X receptor (LXR), and TGR5.

Bile acid Analysis:

Tissues were homogenized; and bile acids were extracted by sequential reflux in 80% methanol and chloroform/methanol (1:1, by vol). An aliquot (equivalent to 1/100 - 1/500th) of this sample extract was taken and the internal standard nordeoxycholic acid (10-20 ug) added. Bile acids were separated into groups based upon their state of conjugation using the lipophilic anion exchange gel, diethylaminohydroxypropyl Sephadex LH-20 (Lipidex DEAP Instruments, Groningen, The Netherlands).

Western Blotting:

Intestinal segments were homogenized in RIPA buffer with protease inhibitors (Sigma, St. Louis, MO, USA). Equal samples were loaded on a 4–12% SDS-polyacrylamide gel (Invitrogen, Carlsbad, CA, USA). ASBT antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and GAPDH antibody was used with their respective secondary antibody conjugated with horseradish peroxidase. Immunopositive bands were visualized by chemiluminescence (Amersham ECL plus detection kit, GE Healthcare, Chalfont St Giles, UK). Density was determined using FujiFILM LAS-4000 Multi Gage V.3 software.

Energy Expenditure Studies:

Total energy expenditure was monitored using a combined open-circuit indirect calorimetry system (TSE Systems Midland, MI). After adaptation for 20 h, the volumes of oxygen (VO2, ml/h) consumed were measured every 45 min for a total of 72 h. Simultaneously, home-cage locomotor activity was determined by using a multi-dimensional infrared light beam system with beams installed on cage-bottom and cage-top levels and activity being expressed as beam breaks. This system also provided data on meal number per day. The rats were placed in the calorimetry system cages during the 5^{th} week post surgery.

Supplementary Data:

Meal Number and Energy Expenditure:

Total daily meal number was increased in the IIS rats ($p<0.05$). Locomotor activity was similar in SH-PF and IIS rats (data not shown). RT PCR in brown adipose tissue for uncoupling protein 1 (UCP1) and the bile acid responsive thyroid hormone G protein coupled receptor (TGR5) revealed no difference between IIS and SH-PF (data not shown).

Figures:

Figure S1

Rats in the ileal interposition (IIS) and Sham pair fed (SH- PF) surgery group loose more body weight initially than the Sham group. N for groups IIS=6, SH=7, SH-PF=8.

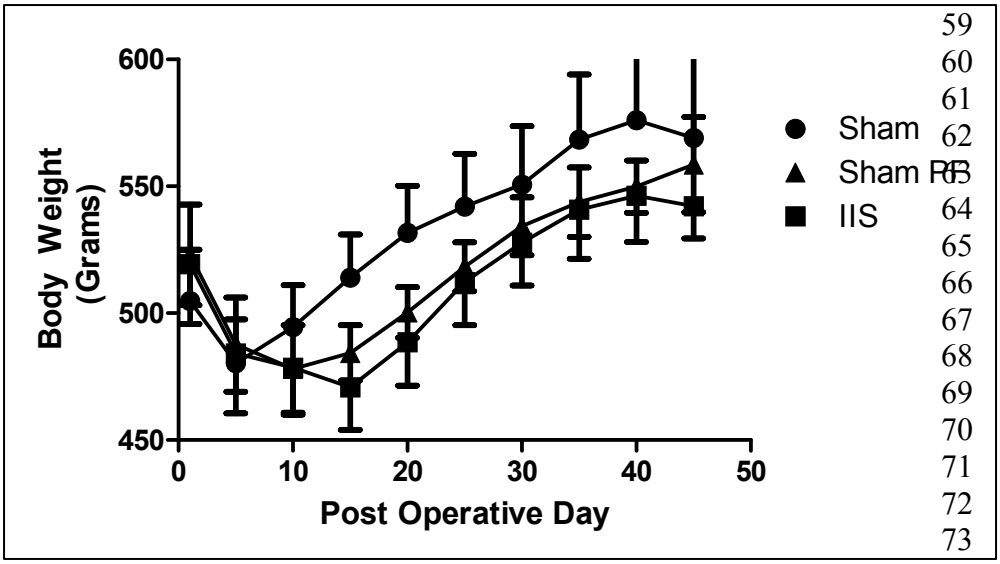
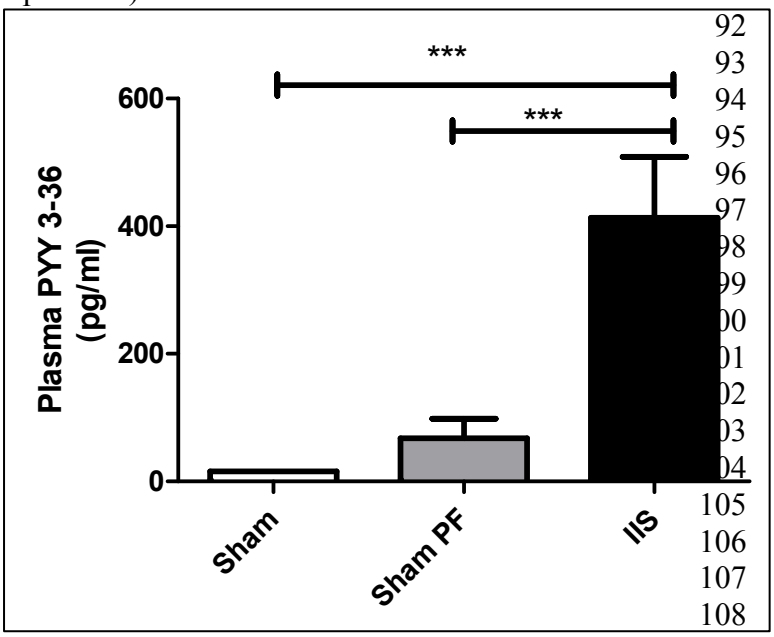


Figure S2: IIS produces exaggerated intestinal hormonal response.

A) *PYY response.*

Plasma active Peptide YY_{3-36} (PYY) levels were measured at 45 and 30 minutes post fixed meal respectively. PYY levels were significantly higher in rats that were in the IIS group compared to SH or SH-PF groups. N for groups IIS=6, SH=7, SH-PF=8. (*** =p<0.001).



B) *GLP-1 response.*

Plasma active Glucagon like Peptide-1 (GLP-1) levels were measured at 45 and 30 minutes post fixed meal respectively. GLP-1 levels were significantly higher in rats that were in the IIS group compared to SH-PF. N for groups IIS=6, SH-PF=8. (* = p<0.05).

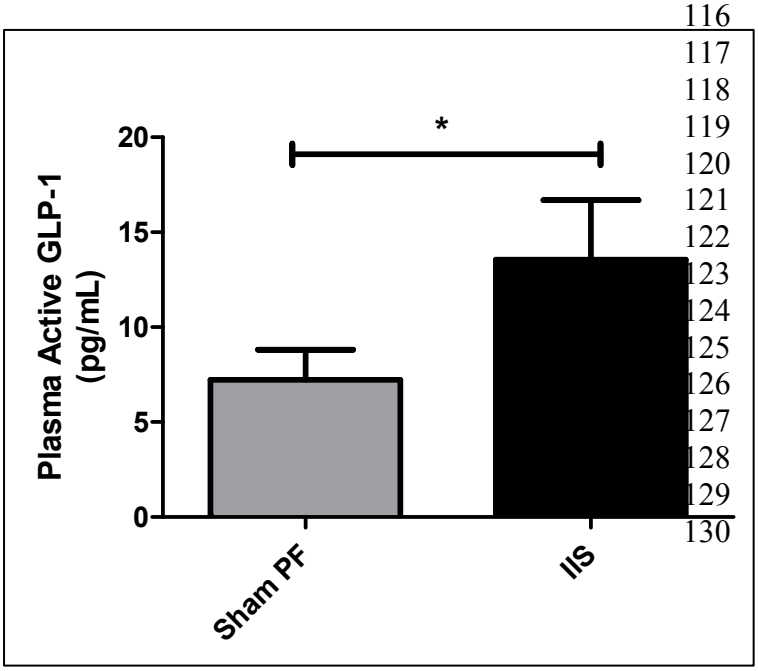
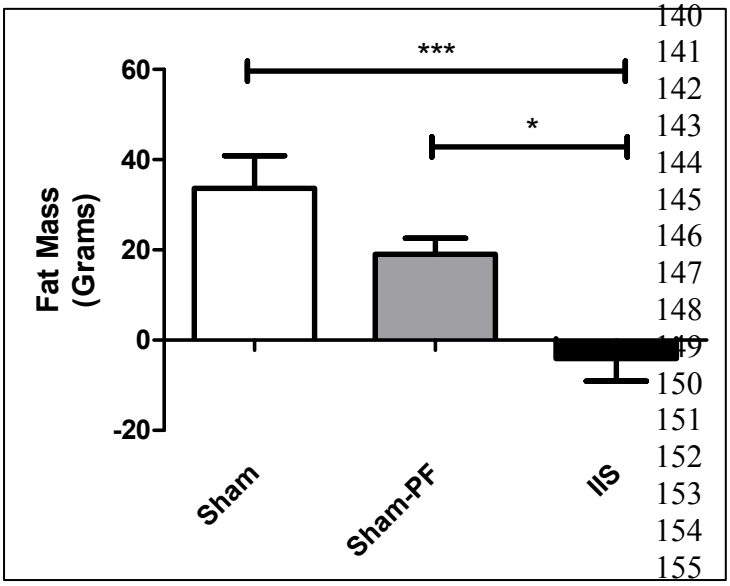
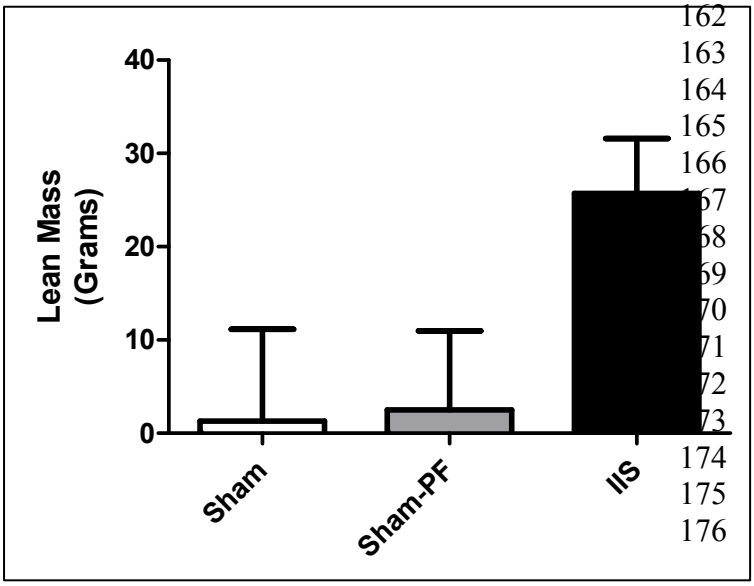


Figure S3: IIS produces fat mass loss, increased energy expenditure, and increased meal number.

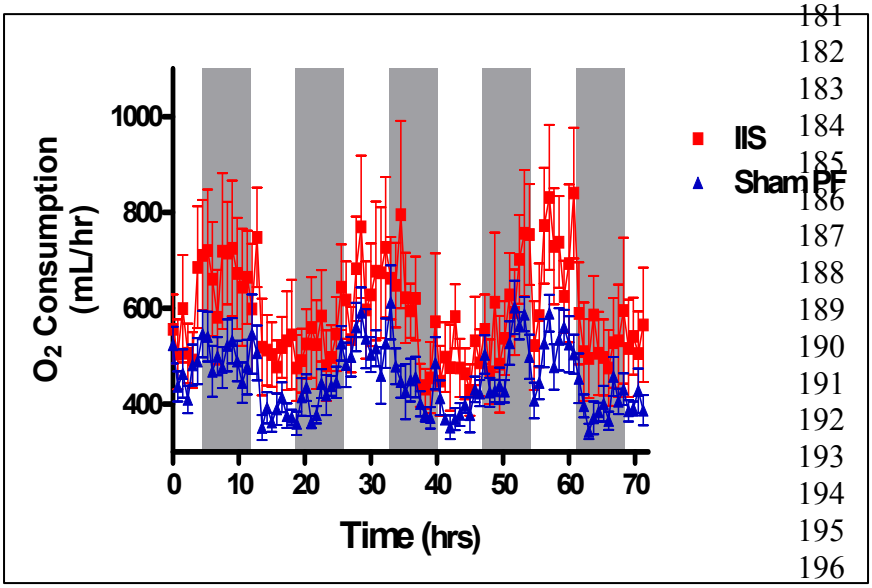
A) *Magnetic resonance for fat mass estimation before surgery and prior to sacrifice*. Rats in the IIS group lost more fat mass after surgery compared to SH and SH-PF who gained fat mass. N for groups IIS=6, SH=8, SH-PF=8. (* = $p<0.05$; *** = $p<0.001$).



B) *Magnetic resonance for lean mass estimation before surgery and prior to sacrifice*. Rats in the IIS group had a trend to gain more lean mass after surgery compared to SH and SH-PF. N for groups IIS=6, SH=8, SH-PF=8.



C) *Oxygen consumption for IIS and SH-PF rats post surgery.*
Rats in the IIS group consume more oxygen compared to SH- PF surgery group. N for groups IIS=7, SH-PF=7.



D) *Meal number per day for IIS and SH-PF rats post surgery.*
Rats in the IIS group consume more meal per day compared to SH- PF surgery group. N for groups IIS=7, SH-PF=7. (*=p<0.05)

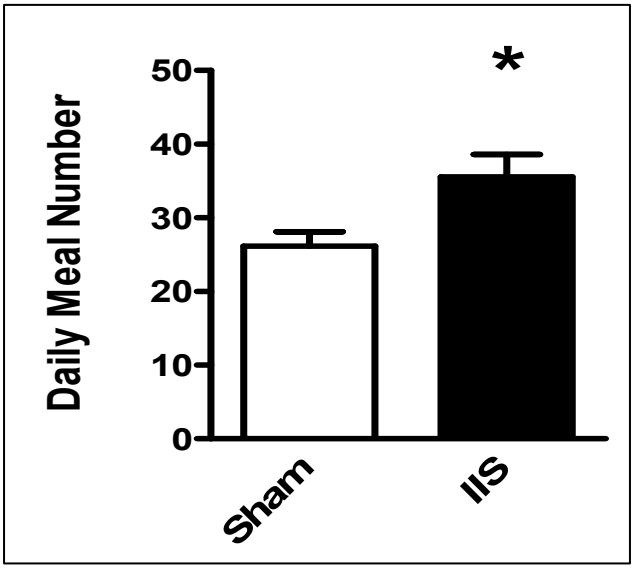


Figure S4: The mRNA expression of Cytochrome P450 genes CYP7A1, CYP8B1 regulating bile acid production and bile acid response elements farnesoid X receptor (FXR) and downstream target short heterodimer protein (SHP) were not significantly different between groups. Genes responsible for bile acid hepatocyte uptake and export were also unchanged (NTCP, BSEP). N for groups IIS=7, SH=8, SH-PF=8.

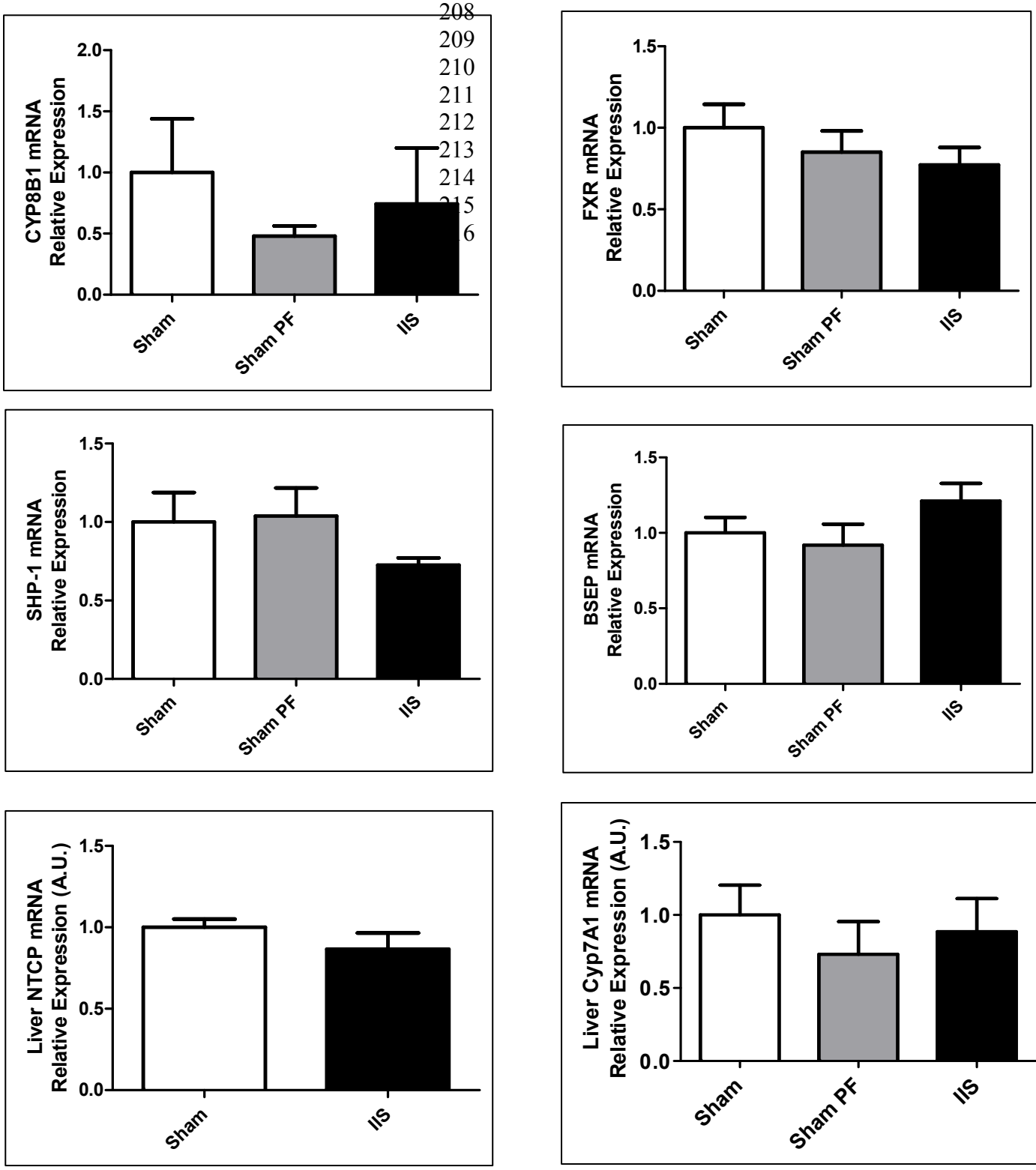


Figure S5: A 72-h fecal collection was performed during the 5th week post-surgery. Fecal cholesterol concentration, as measured by gas chromatography, was not different between the IIS and SH rats. N for groups IIS=7, SH=8.

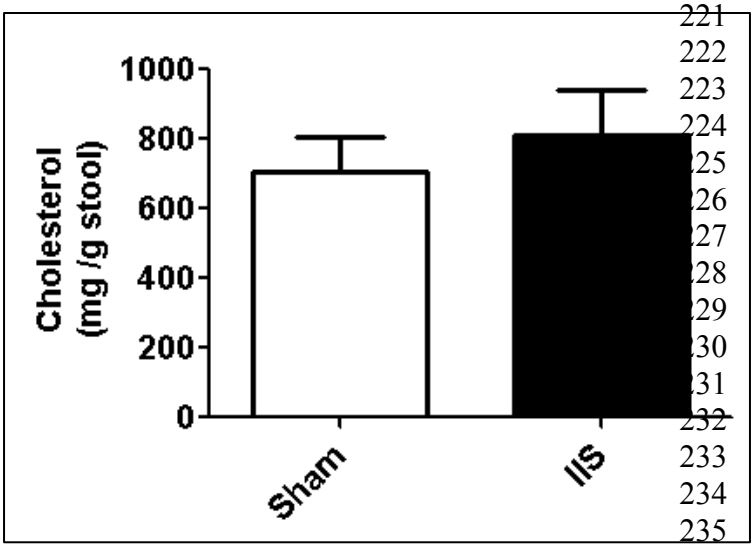


Figure S6: The mRNA expression of Ileal bile acid transport pathway elements farnesoid X receptor (FXR) was decreased in the IIS rats but the downstream target bile salt export pump (BSEP) was not significantly different. N for groups IIS=7, SH=8, SH-PF=8.