TARGETED INTESTINAL OVER-EXPRESSION OF THE IMMEDIATE EARLY GENE TIS7 IN TRANSGENIC MICE INCREASES TRIGLYCERIDE ABSORPTION AND ADIPOSITY

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Summary
Following loss of functional small bowel surface area due to surgical resection, the remnant gut undergoes an adaptive response characterized by increased crypt cell proliferation and enhanced villus height and crypt depth, resulting in augmented intestinal nutrient absorptive capacity. Previous studies showed that expression of the immediate early gene Tis7 is markedly upregulated in intestinal enterocytes during the adaptive response. To study its role in the enterocyte, transgenic mice were generated that specifically overexpress TIS7 in the gut. Nucleotides −596 to +21 of the rat liver fatty acid binding protein promoter were used to direct abundant overexpression of TIS7 into small intestinal upper crypt and villus enterocytes. TIS7 transgenic mice had increased total body adiposity and decreased lean muscle mass compared to normal littermates. Oxygen consumption levels, body weight, surface area, and small bowel weight were decreased. On a high fat diet, transgenic mice exhibited a more rapid and proportionately greater gain in body weight, with persistently elevated total body adiposity, and increased hepatic fat accumulation. Bolus fat feeding resulted in a greater increase in serum triglyceride levels, and an accelerated appearance of enterocytic, lamina propria, and hepatic fat. Changes in fat homeostasis were linked to increased expression of genes involved in enterocytic triglyceride metabolism, and changes in growth with decreased IGF-1 expression. Thus, TIS7 overexpression in the intestine altered growth, metabolic rate, adiposity, and intestinal triglyceride absorption. These results suggest that TIS7 is a unique mediator of nutrient absorptive and metabolic adaptation following gut resection.
INTRODUCTION

The small intestine contains a dynamic epithelium that can rapidly adapt to changes in its luminal environment. Following loss of functional small bowel surface area resulting from small bowel resection, crypt cell proliferation is stimulated in the remnant gut and this contributes to an adaptive response characterized by enhanced villus height, crypt depth and increased nutrient absorptive capacity. To identify the mechanisms responsible for adaptation, we cloned intestinal genes that are differentially expressed in a rat small bowel resection model of adaptation (1). Murine Tis7 (homologous to rat PC4) was one of several genes that showed increased mRNA expression in the remnant small bowel during the intestinal adaptive response following surgical resection in rodents. The TIS7/PC4 orthologue is expressed in the adapting gut in upper crypt and villus-associated small bowel enterocytes (2).

The TIS7/PC4 orthologue is an immediate early gene that is upregulated in response to growth factors (3-5). It encodes a plasma membrane bound protein that translocates to the nucleus upon growth factor stimulation (6), and following c-Jun activation in epithelial cells (7). We have previously shown that its expression increases in intestinal epithelial cells in response to a variety of growth factors, including glucagon-like peptide 2 (GLP-2) and EGF (8). TIS7 has been shown in cultured cells to function as a transcriptional co-regulator (7,9), and TIS7 null mice have defective muscle regeneration after injury (10). By 24 months of age, these mice have significantly decreased body weight due, at least in part, to decreased skeletal muscle mass. No specific intestinal or other phenotype was noted but intestinal morphology and response to injury and/or resection were not examined.

To investigate the role of TIS7 in the normal and adapting murine intestine, we generated transgenic mice that overexpress this gene in enterocytes of the small intestine and colon, thus mimicking the pattern of gene expression seen during adaptation to resection. We used promoter sequences from the rat liver fatty acid binding protein gene (Fabpl-596 to +21) to direct high levels of target gene expression to the crypt and villus epithelium of the proximal small bowel, with lower levels of transgene expression in the distal small bowel and proximal colonic epithelium (11-13). TIS7 transgenic mice exhibited a distinct phenotype. As early as postnatal day 4, they weighed less than wild type littermates. By 1 month, they exhibited decreased total body surface area, but had increased adiposity and decreased lean body mass. TIS7 overexpression also decreased small bowel length and villus height. Despite these changes, triglyceride absorption was enhanced in the transgenic gut, and following small bowel resection, the transgenic mice exhibited an intact morphologic adaptive response. Our data indicate that TIS7, induced in the gut epithelium in response to nutrient stress and deprivation, has direct effects on enterocytic fat absorption and thus acts to enhance functional adaptation following resection. These results also suggest that modulation of fat metabolism in the gut can affect whole body metabolic rate and adiposity/obesity.

EXPERIMENTAL PROCEDURES

Animals: FVB/N mice were housed in Washington University School of Medicine animal facilities and were maintained on a strict 12 h:12 h light/dark cycle. Animals were fed a standard rodent chow diet (Picolab 20, Ralston Purina, St. Louis MO, containing 4.5, 20.0 and 36.8g/100g from fat, protein and carbohydrate, respectively) except as described below for the fat feeding and small intestinal resection experiments. The Animal Studies Committee of the Washington University School of Medicine approved all animal experimentation. Mice were sacrificed at various time points and small intestines were collected, rinsed and weighed, and length measured by suspension with a fixed weight. Intestines were then divided into four segments including duodenum, proximal jejunum, distal jejunum, and ileum. All other organs were harvested and weighed. Tissues were frozen in liquid nitrogen for RNA and protein isolation, and placed in optimal cutting temperature solution (OCT) and in formalin for histochemical and immunohistochemical analysis.

Generation of TIS7 transgenic mice:
Nucleotides –596 to +21 of the liver fatty acid binding protein (Fabpl) gene (gift of Jeffrey Gordon, Washington University) were utilized to direct abundant expression of TIS7 into the crypt and villus epithelial cells of rodent small intestine (11). Fabpl -596 to +21 was fused to the full length mouse TIS7 cDNA, generated by RT-PCR of mouse intestinal RNA (Figure 1A). The Tis7 cDNA sequence was confirmed by automated sequencing analysis performed by the Protein Chemistry Facility of Washington University
School of Medicine. The full length cDNA was inserted into the BamHI site of the vector pLPNDon. Twenty spacer nucleotides were placed between the Fabpl promoter and the ATG of Tis7 to ensure efficient transcription. In addition, nucleotides +3 to +2150 of the human growth hormone (hGH) gene were linked to the 3’ end of tis7 cDNA, to ensure appropriate processing of the transgenic mRNA. The insertion of three termination codons between the Tis7 cDNA and hGH+3 to +2150 sequences and the lack of an initiation codon and internal ribosomal entry site in the hGH+3 to +2150 sequence prevents translation of hGH transcripts. The transgene was excised from the vector by Not1/Sal1 digestion, and the purified construct was injected into the pronuclei of FVB/N oocytes, courtesy of the Animal Models Core of the Digestive Diseases Research Core Center. Four transgenic founders were generated, and experiments were performed using two independent transgenic lines derived from two founders (#2412 and #2454).

**Screening of transgenic mice:** The presence of the transgene was determined by RT-PCR of tail genomic DNA to detect a portion of the hGH gene. The forward 5’ hGH oligonucleotide primer is 5’-CTG CAC CAG CTG GCG TTT GAC ACC TAC CAG-3.’ The reverse 3’ hGH oligo primer is 5’-TTT CTG TGT TTC CTC CCT GTT GGA GGG-3’. RT-PCR was performed using Klentaq LA polymerase.

**Growth curves and body composition analyses:** For analysis of growth rates, transgenic mice and their normal littermates were weighed every 2-3 days after birth. Body composition analyses were performed on anesthetized mice by dual-energy X-ray absorptiometry (DEXA), using a small animal densitometer (PIXImus, Lunar Instruments, Fitchburg, WI) as described in (14). Body surface area was calculated by the DEXA measurement. **Indirect Calorimetry:** Animals were studied following sedation, at rest and in a fed state, in a single-chamber indirect calorimetry Oxymax system to measure oxygen consumption and carbon dioxide production (Columbus Instruments, Columbus, Ohio) as per (14,15). Transgenic mice and normal littermates were studied at room temperature. **Feeding studies:**

**a. Quantitative food consumption study:** Mice from both transgenic lines #2412 and #2454 and normal littermates were caged individually in metabolic cages containing inserts that permit collection of wasted food. Six week old and 2 month old transgenic mice and wild type littermates were studied. The quantity of food ingested per mouse was measured every 2 days for 10-14 days (n=4 per group).

**b. High fat feeding experiment: chronic** Transgenic mice and normal littermates were maintained for 14 days on a synthetic, high fat diet (21% weight/weight, with 42% of total calories from fat; Adjusted Calories Diet TD 88137,Harlan Teklad, Madison, WI). Mice were weighed on days 2, 5, 10 and 14 after beginning the high fat diet, and sacrificed on day 14 (n=4 per group, for males and females). Livers and intestines were harvested for Oil Red O staining.

**c. Intestinal triglyceride absorption in vivo: acute high fat feeding** Experiments were performed as described (16). Transgenic mice and normal littermates were fasted overnight and serum removed for fasting triglyceride and free fatty acid levels. Mice received a bolus (500 µl by gavage) of corn oil, and were sacrificed at either 1h (WT, n=4; TG, n=3) or 3h (WT, n=5; TG, n=3) after gavage feeding. Serum triglyceride, free fatty acid and cholesterol levels were measured, and tissues harvested for histochemical analysis. Routine hematoxylin and eosin staining, Oil Red O staining to detect fat, and electron microscopy were performed.

**Fecal fat quantitation:** Mice were caged individually in metabolic cages with inserts that permit collection and prevent ingestion of fecal material. Mice were maintained either on a normal chow diet or high fat diet for 14 days and feces were collected every two days. Fecal fat was quantified by extraction with 2:1 chloroform:methanol as per (17).

**Intestinal resection surgery and tissue harvesting:** Three month old TIS7 transgenic mice and normal littermates were anesthetized with ketamine HCl (87 mg/kg)/xylazine HCl (13 mg/kg) and isoflurane inhalation, and underwent 50% bowel resection, beginning 2-3 cm distal to the ligament of Treitz and extending to 8 cm proximal to the ileocecal valve, as previously described (19). An end-to-end anastomosis was formed between the remnant jejunum and ileum, using 9-0 silk sutures. All animals received gentamicin (0.2 mg in 0.5 ml saline intraperitoneally) and buprenorphine (.03 mg/kg subcutaneously) for analgesia. Mice were sacrificed at two weeks postoperatively under isoflurane anesthesia, by cervical dislocation. The remnant intestines were divided into duodenal-jejunal and ileal segments, and were either fixed in...
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Microscope. Photographs were recorded with Ultra-Cut, post stained in 4% uranyl acetate and Samples were thin sectioned on a Reichert-Jung dehydrated, and embedded in Polybed 812. tetroxide, tannic acid, and uranyl acetate, then and were sequentially stained with osmium glutaraldehyde in 0.1M sodium cacodylate buffer proliferation is expressed as the percentage of labeled cells per full length crypt, (number of 5-BrdU was detected with a monoclonal anti-BrdU mg/kg), and were sacrificed 90 min after injection. 5-Bromodeoxyuridine (8g/L), total dose 120 of 5-bromodeoxyuridine incorporation into DNA. Mice were injected subcutaneously with a solution into DNA. Mice were injected subcutaneously with a solution of 5-bromodeoxyuridine (6g/L) and 5- fluorodeoxyuridine (0.8 g/L), total dose 120 mg/kg), and were sacrificed 90 min after injection. 5-BrdU was detected with a monoclonal anti-BrdU antibody (Zymed Laboratories, San Francisco CA) and streptavidin-biotin amplification. Crypt cell proliferation was measured by 5-bromodeoxyuridine incorporation into DNA. Electron microscopy: In the acute fat feeding experiments, intestines were fixed at 4°C in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer and were sequentially stained with osmium tetroxide, tannic acid, and uranyl acetate, then dehydrated, and embedded in Polybed 812. Samples were then sectioned on a Reichert-Jung Ultra-Cut, post stained in 4% uranyl acetate and lead citrate, and viewed on a Zeiss 902 Electron Microscope. Photographs were recorded with Kodak EM film. Tissue lipid quantitation: Hepatic lipids were extracted by homogenizing 100-400 mg wet weight of liver in 1.5-2.0 ml PBS and adding 0.1% sulfuric acid as well as 5 ml chloroform:methanol (2:1). The organic phase was collected and treated with chloroform containing 1% Triton X-100 in a glass tube, which was then vortexed and incubated at 37 degrees C for 20 minutes. Triglyceride and cholesterol content were assessed as per (21).

Serum lipid analyses: Serum total cholesterol, and triglyceride concentrations were determined using Cholesterol E, and L-type TG-H kits respectively and serum free fatty acids were determine using the NEFA C kit (Wako Chemicals, Richmond, VA).

Hormone assays: Serum levels of mouse growth hormone, IGF-1, insulin, leptin and glucagon were measured by Ani Lytics, Inc. (Gaithersburg, MD). Serum for growth hormone levels was collected strictly between 0800 and 0830 a.m. Immunoblot and Northern blot hybridization analyses: For immunoblots, a rabbit polyclonal anti-TIS7 antibody was prepared. TIS7 protein was expressed and purified using the IMPACT-CN system (New England Biolabs, Beverly, MA). The mouse Tis7 cDNA was subcloned into the pTYB2 vector and expressed in E coli ER2566 cells. The intein-TIS7 fusion protein was purified after loading onto a chitin bead column, followed by cleavage with DTT. TIS7 protein was run on an SDS PAGE gel to check for the adequacy of purification. A rabbit polyclonal antibody to mouse TIS7 protein was generated by BioDesign (Saco, Maine). Intestinal extracts were prepared and protein aliquots were electrophoresed on 10% SDS-polyacrylamide gels and transferred onto PVDF-plus membranes. Immunoblots were incubated with the rabbit polyclonal anti-TIS7 antibody (1:2000) followed by horseradish peroxidase-conjugated anti-IgG antibodies (1:10,000, Amersham Biosciences Corp, Piscataway, NJ), and developed with chemiluminescent peroxidase substrate (ECL Western blotting Kit, Amersham Biosciences Corp.). For Northern blot hybridization, RNA was purified using TRIzol reagent (Invitrogen) and run on agarose gels. Nylon transfer membranes were probed with a radiolabeled alpha dCTP 32 cDNA probe (RediPrime II, Amersham Biosciences Corp). Membranes were visualized either by exposure of Kodak Biomax MS film or by using a Molecular Dynamics Phosphor Screen, analyzed with Molecular Dynamics software.

Quantitative real time RT-PCR: Total RNA from duodenum, proximal jejunum, distal jejunum, ileum, and liver was extracted by Trizol (Invitrogen, Carlsbad, CA.). The RNAs were treated with DNaseI using the DNA-free kit (Ambion, Austin, TX). First strand cDNA was synthesized from 1 μg of total RNA using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) with random hexamer primers.
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Real-time PCR analysis was performed on SDS 7000 (Applied Biosystems, Foster City, CA) using 2X Sybr Green Master Mix (Applied Biosystems, Foster City, CA). Oligonucleotide primers were chosen using the Primer Express software (Applied Biosystems, Foster City, CA). Primers used in quantitative RT-PCR are TIS7 forward 5'-CGC TGT CTG AAA AAA GGA AAG AGT-3' and reverse 5'-GGC CCA GCT GAA TAC AAA GAA C-3'; IGF-I exon 1 forward 5'-GAT GGG GAA AAT CAG CAG CC-3', exon 2 forward 5'-TGC TGT GTA AAC GAC CCG-3', and common reverse 5'-CAA CAC TCA TCC ACA ATG CGC-3'; apolipoprotein AIV forward 5'-CAA TGC CAA GGA GGC TGT AGA-3' and reverse 5'-AGT TTG TCC TTG AAG AGG GTA CTG-3'; diacylglycerol acyltransferase 1 forward 5'-TCT GCC TCT GGG CAT TC-3' and reverse 5'-GAA TCG GCC CAC AAT CCA-3'; diacylglycerol acyltransferase 2 forward 5'-AGA ACC GCA AAG GCT TTG TG-3' and reverse 5'-AGG AAT AAG TGG GAA CCA GAT CAG-3'; microsomal triglyceride transfer protein forward 5'-AAG ACA GCG TGG GCT ACA AAA-3' and reverse 5'-TCA TCA TCA CCA TCA GGA TTC C-3'; intestinal fatty acid binding protein forward 5'-ACT AAT CCA GAC CTA CAC ATA TGA AGG A-3' and reverse 5'-GCT CCA GGC TCT GAG AAG TTG A-3'; liver fatty acid binding protein forward 5'-GAA CTT CTC CGG CAA GTA CCA A-3' and reverse 5'-GTC CTC GGG CAG ACC TAT TG-3'; cellular retinol binding protein 2 forward 5'-AGA TGA AGG CCC TAG ATA TTG ATT TT-3' and reverse 5'-AGT GAT GAT CTT CGT CTG AGT AGT-3'; sodium/glucose cotransporter 1 forward 5'-CAG CAT ATT GAT CAT CTC CTT CCT-3' and reverse 5'-TGC GAT GAC TCC AAC ACA AAC G-3'; 18S ribosomal RNA forward 5'-CGG CTA CCA GGA GGC TGT AGA-3' and reverse 5'-GGC CCA GCT GAA TAC AAA GAA C-3'.

For all primer sets, the kinetics of the PCR was confirmed by serial dilutions of different cDNA preparations. These analyses verified that the efficiencies of amplification were equal for both primer sets and thereby allowing quantification by the comparative Ct method (User Bulletin #2, Applied Biosystems, Foster City, CA).

Statistical analyses: Means were compared between normal and transgenic mice using a Student's t test. Values in the text are means +/- SEM. Differences were considered significant at p<0.05.
Generation and analysis of transgenic mice with intestine-specific overexpression of TIS7

Transgenic mice were generated that specifically overexpress TIS7 in the intestine, as per Materials and Methods. TIS7 transgenic mRNA levels in gut and other organs were quantified by Northern blot hybridization and quantitative real time RT-PCR assays, and TIS7 protein levels were assessed by Western blotting. Hybridization of total RNA with TIS7 specific cDNA probes detected transcripts of sizes varying from 2.3 kb to ~4.3 kb in the intestines of transgenic mice, while native TIS7 mRNAs of 2.3 kb were detected in wild type mice as expected (Figure 1B). Several higher molecular weight TIS7 transcripts were produced by alternative splicing of hGH, as found for other transgenic mouse constructs utilizing the hGH gene to ensure appropriate RNA processing (T. Simon, Washington University, personal communication). Intestines of transgenic animals typically presented a cephalocaudal gradient of TIS7 expression; steady state mRNA levels were increased in duodenum, jejunum and ileum compared to wild type littermates, with the greatest increase in expression in duodenum (Figure 1C). TIS7 protein levels were also detected in the small intestine of transgenic mice (Fig. 1D). As reported in other transgenic animals containing this promoter, expression in the liver was increased (11), but we found increased liver expression in only one of the two transgenic lines examined. The phenotype of the mice was the same in both lines, irrespective of the liver expression. All experiments described below were performed in both lines. Expression was not increased in the kidney, and TIS7 mRNA levels were unchanged in transgenic compared to wild type mice in various other non-digestive organs, including heart, lung, skeletal muscle, white adipose tissue and brain (data not shown).

Reduced body weight and small bowel weight, and increased adiposity in TIS7 transgenic mice

Transgenic mice appeared healthy without excess mortality for up to 1 year of observation. However, TIS7 overexpression resulted in several phenotypic changes. Small bowel weight was significantly reduced in TIS7 mice compared to normal littermates, but the weights of other organs were unchanged (Fig. 2A). TIS7 transgenic mice showed reduced total body weight, from postnatal day 4 through the suckling-weaning transition, and into adulthood (Fig. 2B and C, representative

growth curves for males and females from individual litters). Both male and female mice showed body weight differences beginning shortly after birth. Analysis of mice aged 120 days revealed that the weight differences remained constant for males and females (data not shown).

To further evaluate the difference in body weight, food consumption was quantified, organs were harvested and weighed, DEXA analyses were performed to determine body composition, and oxygen consumption was measured to determine basal metabolic rates. To examine the temporal regulation of the phenotypic changes in relation to growth, six week, 2-3 month and 9 month old mice were studied. There were no significant differences in consumption of a normal chow diet, measured for 10-14 days in 6-8 week (data not shown) or 2.5-3 month old male and female transgenic and wild type mice (Fig. 2D). Surprisingly, dual energy X-ray absorptiometry showed that although the TIS7 mice weighed less and had decreased body surface area, they had a higher percentage of body fat and reduced lean body mass compared to wild type littermates (Fig. 2E and Table 1). Body composition was significantly different as early as 6 weeks after birth, and became even more apparent by 3 months after birth (Figure 2E and Table 1). The percentage of body fat mass was increased in both male and female transgenic mice (e.g., at three months, 16% versus 25% for wild type and transgenic mice, respectively). There was no significant difference related to gender in fat mass, in normal or transgenic mice at the ages examined (data not shown). To determine the phenotype in aged mice, DEXA was performed at 270 days; body surface area remained reduced (7.66 cm² vs. 9.85 cm², p<0.000001), yet adiposity was further increased (35%, vs. 22% p<0.00001) and as a result, weights were no longer significantly different. The calculated body mass indices for these aged mice (measured as g/cm²) are 3.89 for TG mice and 3.14 for wild type mice.

To further characterize the differences in weight and body composition in TIS7 transgenic mice, oxygen consumption was assessed as a measure of basal whole body metabolic rate (14). The resting oxygen consumption was significantly reduced in TIS7 transgenic mice compared to wild type mice (Table 1). This difference was also observed when the mice were exercised on a treadmill (data not shown). To determine whether fat and/or carbohydrate oxidation was reduced in these mice, the respiratory quotient was measured by indirect calorimetry and was unchanged (wild
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Type, ratio 0.796 vs. transgenic, 0.781, p=0.3 (NS)). In addition, to determine whether there was altered thermogenesis in transgenic mice, the expression of uncoupling protein 1 (UCP-1) was measured in brown fat by quantitative RT-PCR. UCP-1 mRNA expression was unchanged in the brown fat of normal compared to transgenic mice (data not shown).

**TIS7 transgenic mice fed a high fat diet exhibit rapid weight gain and increased hepatic fat accumulation**

Since TIS7 was specifically overexpressed in gut, to elucidate the etiology of the increase in adiposity in TIS7 transgenic mice, we quantified intestinal fat absorption in TG mice on a standard chow diet and a high fat diet. We hypothesized that TIS7 TG mice might have increased adiposity due to a more rapid absorption of luminal fat compared to normal littermates. On a standard chow diet, transgenic mice had decreased fecal output (0.93g/d vs. 1.16g/d, p=0.055) and had a lower total fecal lipid content (0.136g vs. 0.170g total for 7 days, p<0.01) compared to wild types, suggesting increased fat absorption in the transgenic mice. Mice were then fed a high fat diet (42% of calories from fat) for 14 days. Despite equivalent food consumption (data not shown), TIS7 TG mice gained weight much more rapidly than normal littermates (Fig. 3A, females and Fig. 3B, males). Male and female TG mice began to gain weight by 5 days after initiating the high fat diet, whereas male wild types did not start to gain weight until 10 days after introduction of the high fat diet, and female wild types did not gain at all during the entire two week period. Although there was no change in the gross appearance of the liver in TIS7 TG mice fed a normal chow diet, the livers of TG mice on the high fat diet were paler than the controls. The livers of transgenic and control mice were examined for evidence of fat accumulation. Although transgenic mice on a normal chow diet showed no evidence of fat accumulation, a marked increase in Oil Red O staining was noted in livers of transgenic mice that were fed a high fat diet for two weeks compared to controls, indicating increased fat stores (Fig. 4A,B).

**TIS7 transgenic mice have accelerated enterocytic triglyceride absorption**

Because of the rapid weight gain noted in high fat fed TG mice, we wished to determine the effects of overexpression of TIS7 on enterocytic triglyceride absorption, to address the hypothesis that fat absorption was enhanced in these mice. Mice were fed corn oil by gavage, and were sacrificed at 1 and 3 h after administration of the fat bolus. The TIS7 TG mice showed a marked increase in enterocytic and lamina propria fat accumulation detected by Oil Red O staining of intestines (Fig. 4C, D), compared to fat fed wild type littermates. Livers of wild type and transgenic mice were also stained with Oil Red O, revealing a marked increase in hepatic fat in the bolus corn oil fed transgenic mice (Fig. 4E,F). In addition, the TIS7 TG mice demonstrated increased serum triglyceride (Fig. 5A) and serum free fatty acid levels (Fig. 5B) compared to controls, at 1 hour after gavage. Serum triglyceride and free fatty acid levels were more than two fold higher in the TIS7 TG mice compared to controls. These data suggest that TIS7 TG mice have an accelerated absorption of fat into the enterocyte and across the gut wall, compared to wild type controls.

To further clarify the effects of TIS7 overexpression on enterocytic fat absorption, electron microscopic analysis was performed on the transgenic and wild type mouse intestines from mice that received the high fat bolus feed (proximal jejunum, Fig. 6). Consistent with the results obtained by Oil Red O staining, enterocytes and the lamina propria were filled with fat droplets at 1 hour after gavage in transgenic mice (Fig. 6B,D, and F), compared to wild type control mice (Fig 6A,C, and E). This difference was most remarkable in the proximal small bowel (duodenum-jejunum). Both large and small vesicular fat droplets were noted in the transgenic mouse enterocytes. These results support the hypothesis that fat absorption is accelerated in the proximal transgenic mouse gut.

**TIS7 transgenic mice show increased intestinal expression of genes involved in triglyceride metabolism**

Since intestinal fat absorption and transport out of the enterocyte and small bowel, appeared enhanced in TIS7 TG mice, the expression of genes involved in intestinal absorption of triglycerides and other nutrients were analyzed in WT and TG mice on a normal chow diet, using quantitative real time RT-PCR. Diacylglycerol acyltransferases DGAT1, DGAT2, and microsomal triglyceride transfer protein (MTP) mRNA levels were increased in the TIS7 TG whole intestine (Table 2), and the maximal increase in expression for each gene was found in duodenum, the site exposed to the highest intraluminal fat concentration. On a high fat diet, MTP, DGAT1 and DGAT2 mRNA levels were also increased in the TG mice compared to
Hepatic levels of IGF-1 transcripts initiated from exon 1 and exon 2 (22) were quantified. mRNA levels of the two major isoforms, initiated in intestine, by quantitative real time RT-PCR. IGF-1 mRNA expression was also determined in mice specifically overexpress TIS7 in the gut, quantified in TG and WT liver. Because TIS7 TG (23); thus hepatic IGF-1 mRNA levels were the liver is a major source of circulating IGF-1 in many tissues, but is most abundant in liver, and the early postnatal period (22). IGF-1 is expressed GH independent in postnatal day 4, GH-independent regulation of IGF-1 expression was likely in the TIS7 transgenic mice.

Serum IGF-1 levels are altered in TIS7 transgenic mice

The phenotypic alterations in the TIS7 transgenic mice included reduced body weight and body surface area as well as increased adiposity. As putative factors responsible for these changes, serum levels of growth regulatory hormones including insulin, glucagon, insulin-like growth factor-1 (IGF-1) and growth hormone (GH) were measured at 1 month of age (during rapid growth) and 3 months of age (at maturity, Table 3). TIS7 transgenic mice had significantly although modestly reduced serum IGF-1 levels compared with wild type mice. Serum IGF-1 levels of 1-month and 3-month transgenic mice were 61% and 74% that of control mice, respectively. Serum glucagon levels were unchanged at 1 month of age but were significantly higher in TIS7 transgenic mice at 3 months, and fasting blood glucose levels were modestly reduced in 3 month old transgenic mice (Table 3). In addition, the transgenic mice had increased serum leptin levels compared to the control mice at 3 months, likely reflecting the increase in adiposity. No significant difference was found in serum insulin or GH levels.

Because GH levels were unchanged, and differences in growth were present as early as postnatal day 4, GH-independent regulation of IGF-1 expression was likely in the TIS7 TG mice. IGF-1 expression in the liver is GH independent in the early postnatal period (22). IGF-1 is expressed in many tissues, but is most abundant in liver, and the liver is a major source of circulating IGF-1 (23); thus hepatic IGF-1 mRNA levels were quantified in TG and WT liver. Because TIS7 TG mice specifically overexpress TIS7 in the gut, IGF-1 mRNA expression was also determined in intestine, by quantitative real time RT-PCR. mRNA levels of the two major isoforms, initiated from exon 1 and exon 2 (22) were quantified. Hepatic levels of IGF-1 transcripts initiated from

Decreased villus height and small intestinal length in TIS7 transgenic mice

To evaluate the change in small bowel weight and to determine the effect of TIS7 overexpression on small bowel morphology, epithelial cell differentiation, small bowel lengths, villus heights, and crypt depths were measured in transgenic mice and controls, in duodenum, proximal and distal jejunum and ileum (Fig. 7). All four epithelial cell types were present, including enterocytes, goblet cells, enteroendocrine cells and Paneth cells. Muscle thickness was unchanged. Although villus and crypt morphology appeared normal, villus heights were decreased in all segments except the distal bowel (ileum, Fig. 7A). Crypt depths were unchanged in all segments of the gut (Fig. 7B). Total small bowel length was also significantly reduced in transgenic mice compared to controls (Fig. 7C), and there was a greater reduction in length at three months after birth compared to small bowel length at one month postnatal. Crypt cell proliferation and apoptotic rate in the crypts were unchanged in transgenic mice (data not shown). Apoptotic cells could not be detected on the villus by routine hematoxylin and eosin staining.

The morphologic intestinal adaptive response is intact in TIS7 transgenic mice

To determine whether the overexpression of TIS7 in the intestine altered the morphologic adaptive response following small bowel resection, transgenic and control mice were subjected to 50% bowel resection and animals were sacrificed at 2 weeks postop. Standard measurements of the intestinal adaptive response, including villus heights, crypt depths, 5-bromodeoxyuridine staining to measure crypt cell proliferation, and apoptotic rates were performed. Villus heights in the remnant gut increased following resection, in both TIS7 transgenic mice and control littermates (Fig. 7D). However, in the
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transgenic proximal intestine, the adaptive villus height was lower compared to wild type proximal intestine. As noted in Fig. 7A, villus heights in the proximal gut were significantly lower in TIS7 transgenic mice compared to controls, at baseline prior to resection. Villus heights in the TG mice remained lower post-resection, although both normal and transgenic mice exhibited a similar percentage increase in villus height after resection (37% in wild type and 30% in transgenic mice, Fig. 7D). Crypt depth and crypt cell proliferation increased following resection in both wild type and transgenic mice, but there was no further increase in crypt cell proliferation in the TIS7 TG mice (data not shown). Apoptotic rates were unchanged in operated TG vs. operated wild type mice (data not shown). These results indicate that the adaptive response is intact and unaltered in TIS7 transgenic mice.

DISCUSSION

To begin to elucidate the role of TIS7 in the normal intestine and in the adaptive response following loss of functional small bowel surface area, transgenic mice were generated that overexpress TIS7 in the upper crypt and villus epithelial cells of the intestine. These mice exhibited several unique phenotypic manifestations. Direct intestinal effects of TIS7 overexpression included decreased villus height, small bowel length, and small bowel weight. The mice also showed a striking alteration in body size and composition, with decreased body weight and decreased body surface area, decreased whole body metabolism, yet also increased adiposity and decreased lean muscle mass. Although the morphologic intestinal adaptive response was unaltered in TIS7 TG mice, functional adaptation increased, as manifested by increased triglyceride absorption across the gut wall (Figs. 5 and 6). These results suggest that TIS7 expression increases during nutrient stress/deprivation, as an adaptive mechanism to enhance nutrient absorption and whole body energy flux. Intestinal TIS7 overexpression is also linked to decreased growth, altered energy metabolism, and increased fat storage, all of which are advantageous to the adapting organism following massive gut resection.

The results of several experiments indicate that intestinal overexpression of TIS7 increases enterocytic triglyceride absorption, and acts primarily by affecting triglyceride synthesis and transport out of the enterocyte and across the gut wall. We found that TIS7 transgenic mice had a more rapid and higher percentage weight gain on a high fat diet compared to normal wild type controls. In addition, bolus corn oil feeding produced a marked, early increase in serum triglyceride levels. This was accompanied by enhanced enterocytic and lamina propria Oil Red O staining, and an increased rate of appearance of fat droplets in enterocytes and lamina propria, detected by electron microscopy. Hepatic lipid accumulation also increased in both acute and chronic fat feeding experiments. Finally expression of DGAT1, DGAT2 and MTP mRNA was increased, suggesting that triglyceride synthesis and transport are increased in the TIS7 TG mice. The importance of DGAT1 in chylomicron secretion from the gut has been shown in studies of DGAT1 null mice (25). DGAT1 deficient mice did not malabsorb fat, but instead had reduced postabsorptive chylomicronemia after a fat bolus, and retained fat in enterocytes while on a high fat diet. MTP transports newly synthesized triacylglycerol to apolipoprotein B, in the lumen of the endoplasmic reticulum (26,27), and DGAT2 has been shown to be critical for triglyceride synthesis (28). The increase in gene expression was specific for those involved in triglyceride metabolism and secretion, since we found no change in expression of SGLT-1, the glucose transporter, or CRBPII, a retinol binding protein.

The increase in adipose tissue mass and decrease in oxygen consumption (basal metabolic rate) in TIS7 transgenic mice was an unexpected outcome of intestinal TIS7 overexpression. The increase in adiposity may, at least in part, be explained by the increase in proximal intestinal triglyceride absorption rate, as previously shown in studies of high fat fed, intestinal alkaline phosphatase knockout mice (16). The intestine-specific form of alkaline phosphatase is a brush border protein, regulated by fat feeding and secreted from the enterocyte in response to fat, as part of a surfactant-like particle. It has been postulated to act within the particle to limit the rate of intestinal fat absorption. Like the TIS7 TG mice, intestinal alkaline phosphatase null mice showed a rapid and enhanced weight gain on a high fat diet compared to normal mice; this was proven to be due to an increase in the rate of triglyceride transport from the enterocyte into the bloodstream (16).

The mechanism by which accelerated fat absorption and transport across the gut wall might lead to increased adiposity is unclear. As noted...
Experiments to determine effects on intestinal regeneration after crush injury (10). A role for TIS7 in tissue regeneration was also suggested in the null mice, since young (4 month old) mice have defective muscle regeneration after crush injury (10). Although an intestinal phenotype was not noted, these data suggest that following intestinal resection, increased adaptive gut expression of TIS7 may signal to the body to decrease metabolic rate and increase fat storage through DGAT1.

The increase in intestinal MTP, DGAT1 and DGAT2 mRNA expression may be directly related to TIS7 overexpression, or may be downstream of TIS7, due to changes in the transcriptional regulation and expression of key regulators of these genes. TIS7 has been shown in mammary epithelial cells to act as a transcriptional coregulator (7), interacting with mSin3B and histone deacetylase. In an in vitro model system, specific interactions with the C/EBPα-Sp1 transcription factor module were demonstrated (9). MTP expression is regulated in C/EBPα null mice, (29) and its promoter has a C/EBPα binding site. The DGAT1 and 2 promoters have C/EBPα/Sp1 binding sites (Gene2 Promoter and Model Inspector software, Genomatix Software Inc, Germany). Depending upon the cellular and physiological context, it is possible that TIS7 interacts with these transcription factors to increase rather than suppress gene expression.

Comparison with the TIS7 null mice reveals that, in contrast to the intestinal-overexpressing TIS7 transgenic mice, young TIS7 knockout mice have normal body weights. However, when they are aged to 24 months, their weights are lower compared to wild type litter mates (10). The decrease in body weight was thought to be due to muscle atrophy; however, DEXAs were not performed to precisely delineate body composition and measure adiposity and muscle mass. In addition, a role for TIS7 in tissue regeneration was also suggested in the null mice, since young (4 month old) mice have defective muscle regeneration after crush injury (10). Although an intestinal phenotype was not noted, experiments to determine effects on intestinal regeneration and fat absorption were not performed.

Based on the present studies, it is most likely that TIS7’s function in the intestinal adaptive response is to increase nutrient, and specifically fat absorption, at least in part via transcriptional regulation of intestinal lipid metabolism genes, as indicated above. In the early stages following resection surgery, mechanisms to increase calorie absorption and specifically, transport of lipid out of the intestine in the face of reduced small bowel surface area would be advantageous for survival. TIS7 overexpression in fact did not enhance the morphologic adaptive response (i.e. by further increasing crypt cell proliferation, villus height, and crypt depth) since TIS7 transgenic mice showed a morphologic adaptive response that was equivalent to, but did not exceed, the adaptive response in the wild type mouse, as per Figure 7D. This is consistent with its expression pattern in the gut, which suggests a role in functional adaptation in the enterocyte.

Fat absorption studies were performed using intact intestines in unoperated transgenic mice, and not in mice that were subjected to gut resection. We chose to perform these studies in intact transgenic gut, which already mimicked the adaptive state due to forced TIS7 overexpression. We felt it unlikely that resection would evoke a further increase in TIS7 expression. Also, by performing the absorption studies in TG mice with intact intestines, the confounding factors of short gut and motility changes post-resection could be avoided. In this manner we were able to model the changes in the bowel that resulted from TIS7 upregulation while avoiding altered physiology resulting from resection, that might induce artifactual changes.

The phenotype of the TIS7 TG mice also included a decrease in body surface area and body weight. These changes may have resulted, in part, from the modest decrease in circulating serum levels of IGF-1. IGF-1 null mice that have a complete absence of circulating IGF-1 are severely growth retarded and most die after birth ((30,31)). However, mice with a liver specific IGF-1 knockout showed a marked decrease in circulating IGF-1 (measured at 6 weeks of age) but grew normally (23). In these studies, the albumin promoter was used to express Cre recombainase, which is active by fetal day 19. Despite dramatically lower levels of circulating IGF-1 compared to the TIS7 TG mice, the IGF-1 liver-specific null mice were normal size and weight, and grew normally. These mice were the same
Growth and adiposity in intestinal TIS7 transgenic mice

strain as the TIS7 TG mice (FVB/N). However, others have postulated that in this model, complete deletion of the IGF-1 gene is not achieved until late in postnatal life, thus adequate circulating IGF-1 levels may be present earlier (32). In any event, because the reduction in serum IGF-1 levels in TIS7 TG mice is modest, it is possible that other factors contribute to the observed phenotype of decreased body weight and surface area; however, their identity remains unknown. Serum levels of other hormones that regulate growth, including insulin and GH, were not altered in TIS7 transgenic mice, and TIS7 is not secreted (6,33). Decreased hepatic IGF-1 mRNA levels were found in both transgenic lines (i.e. in lines that did or did not overexpress TIS7 in liver), thus the decrease in hepatic expression is likely to be a secondary effect of overexpression in gut, e.g. perhaps in response to increased hepatic fat accumulation.

Intestinal weight, length and villus height were also decreased in TIS7 TG mice. Intestinal muscle layer thickness appeared unaffected by TIS7 overexpression, suggesting an effect primarily on the epithelium. The factors that determine villus height have not been precisely defined, but crypt cell proliferation rates, epithelial cell migration from crypt to villus tip, and crypt and villus epithelial cell apoptosis (anoikis) all likely play a role. IGF-1 has a potent trophic effect in the gut epithelium (34,35), manifested by increased crypt cell proliferation; thus the decrease in local intestinal IGF-1 production as well as decreased circulating levels may at least in part explain these changes. However, we were unable to show a decline in basal crypt cell proliferation rate by 1 month after birth, or following intestinal resection. The fatty acid binding promoter is active beginning on ~fetal day 16-17 at the time of villus morphogenesis (36), thus detailed studies of prenatal and early postnatal proliferation may provide some clues regarding the underlying mechanisms. Overexpression of TIS7 may also have a direct negative effect on growth locally in the gut, in prenatal or early postnatal life. Following intestinal resection, the residual small bowel shows a marked upregulation in TIS7 mRNA expression (2,37). This increase in expression may also act as a “brake” against unrestrained growth that might occur in the post-resection adaptive gut, thus averting the production of a hyper-proliferative, pre-cancerous state. We also found no change in crypt apoptosis and could not detect changes in villus epithelial cell shedding, or anoikis, by hematoxylin and eosin staining; again pre- and early postnatal studies of apoptosis may also prove fruitful.

In summary, TIS7 appears to play a unique role in the gut adaptive response following loss of small bowel surface area, increasing functional adaptation by enhancing intestinal lipid absorption. TIS7 may act as a gut sensor of nutrient deprivation that signals to the body to reduce whole body metabolism and increase adiposity for energy storage. Future analyses of gut adaptation and triglyceride absorption and transport in Tis7 null mice are likely to provide further insight into TIS7’s role in the gut. Finally, the TIS7 TG mouse model may also prove fruitful for examining the potential link between intestinal lipid absorption, whole body adiposity, and hepatic lipid accumulation or fatty liver, a common complication of obesity in humans (38,39).
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Footnotes:
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Figure 1. Characterization of TIS7 expression levels in TIS7 transgenic (TG) mice. A. Schematic representation of the Fabpl<sup>396</sup>-<sup>21</sup>Tis7-<em>hGH</em> construct for production of gut-specific TIS7 TG mice. B. Detection of endogenous and transgenic TIS7 mRNA in the intestine by Northern blot hybridization analysis, as per Materials and Methods. A representative Northern blot is shown. C. Quantification of regional intestinal expression of TIS7 mRNA in WT (n=3) and TIS7 TG (n=6) mice, by quantitative real-time RT-PCR assays as per Materials and Methods. D. Detection of TIS7 protein in the intestine of TIS7 TG mice by immunoblot assays. A representative Western blot is shown.

Figure 2. Organ weights, growth curves and metabolic studies of wild type vs. transgenic (TG) mice. A. Organ weights were measured in normal (n=9) and transgenic (n=7) mice and data are expressed as means +/- S.E.M. *p<0.03. B,C. Representative growth curves from two litters of TIS7 transgenic mice. Mice were weighed every three to five days. B. Male mice, n = 4 WT and 2 TG. * p<0.03. In litters with more male TG mice, TG weights remained significantly less than normal littermates for up to 120 days. C. Female mice, n=5 WT and n=4 TG. *p<0.05 +p=0.053; #p=0.09. D. Food consumption was measured in 2.5 month old male (n=3 WT and n=3 TG) and female (n=2 WT and n=4 TG) mice. Mice were caged individually with metabolic inserts and the amount of food consumed was measured every 2 days for 14 days. The total amount of food consumed (grams) over 14 days is depicted. E. Body composition was determined by dual energy X-ray absorptiometry (DEXA) and adiposity expressed as fat (% total body mass) in 1.5 month old mice, n=8 WT, n=8 TG; and 3 month old mice, n=14 WT, n=15 TG. *p<0.005, **p<0.00001.

Figure 3. Enhanced body weight gain in transgenic mice following high fat diet feeding. Female (panel A) wild type and transgenic mice and male (panel B) wild type and transgenic mice were fed a high fat diet (42% total calories as fat) for 14 days (n=4 per group for all groups). Data are expressed as the mean percentage of body weight gain, measured at 2, 5, 10 and 14 days after starting the diet. A. *p<0.03; **p<0.05, +P=0.056. B. *p<0.04; **p<0.02.

Figure 4. Increased hepatic and enterocytic fat accumulation in high fat fed TIS7 transgenic mice. A,B. Representative sections of Oil Red O stained livers from mice fed a high fat diet (42% of calories from fat) and sacrificed after 14 days (as per Materials and Methods). A. Wild type liver. B. TIS7 transgenic liver. C,D,E,F. Oil Red O staining of jejunum (C,D) and liver (E,F) from wild type (C,E) or transgenic (D,F) mice gavage fed a corn oil bolus and sacrificed one hour later. Small bowel enterocytes and underlying lamina propria and hepatocytes show markedly enhanced Oil Red O staining, consistent with increased enterocyte triglyceride absorption and transport across the small bowel wall. (All magnifications X 200).

Figure 5. Increased serum triglyceride levels in bolus fat fed TIS7 transgenic mice. Mice were gavage-fed a corn oil bolus and were sacrificed at 1 and 3 hours after feeding. Serum triglyceride (TG, panel A) and free fatty acid levels (FFA, panel B) were measured. Serum TG and FFA levels were markedly increased in male TIS7 transgenic mice at one hour after infusion. (n=3 WT and n=3 transgenic at 1 h, n=4 WT and n=3 transgenic at 3h, *p=0.05 at 1h for TG and FFA levels, p=0.067 at 3h for serum TG, p=NS at 3 h for serum FFAs.). An additional 4 female WT and 8 female transgenic mice were studied at 1 and 3 h with similar results (p=0.03 at 1h for serum TG, p=0.009 at 1h for FFAs, p=NS at 3 h for TG, p=0.05 for FFAs at 3h).

Figure 6: Electron microscopic analysis of proximal jejunum from high fat bolus fed TIS7 transgenic vs. WT mice. Mice were gavage-fed a corn oil bolus as per Fig. 4. Tissues harvested at 1 hour after feeding were fixed in glutaraldehyde and osmium tetroxide/tannic acid to preserve lipid droplets, as per Materials and Methods. A. Wild type jejunum. B. TIS7 transgenic jejunum. The clear spaces represent fat droplets. Note the presence of fat in the lamina propria. C,D. Higher power view of jejunum from wild type (C) and transgenic (D) mice. E. Male wild type jejunum. F. Male transgenic jejunum. A-B 1cm=1.8 microns; C-F 1cm=1.3 microns.
Figure 7. Morphometric analysis of wild type and transgenic intestine prior to and following 50% intestinal resection: A,B. Intestines were harvested and divided into four segments including duodenum, proximal jejunum, distal jejunum and ileum. Villus heights (A) and crypt depths (B) were measured as per Materials and Methods (n=12 WT and n=11 TG mice, *p=0.05). C. Small bowel lengths were measured in 1 month or 3 month old wild type or TIS7 TG mice. (n=8 WT and TG mice, 1 month; n=15 WT and n=14 TG, 3 month, *P<0.01). D. Morphometric analysis of villus heights either before (white and striped bars) or after surgical resection (stippled and black bars), in wild type (white and stippled bars) and transgenic (striped and black bars) mice. Data are expressed as means +/- S.E.M. *p<0.01 unoperated compared to operated; +p<0.03 wild type operated vs. transgenic operated; ++p<0.03 wild type unoperated compared to transgenic unoperated.
### Table 1

|                   | Control          | Transgenic       | P value       |
|-------------------|------------------|------------------|---------------|
| Weight (g)        | 25.59 +/- 0.81   | 21.2 +/- 0.59    | 0.00045 (<0.001) |
| Body surface area (cm²) | 9.86 +/- 0.27   | 7.07 +/- 0.16    | 8.62X10⁻¹⁴          |
| Fat (g)           | 4.093 +/- 0.29   | 5.4 +/- 0.33     | 0.0069 (<0.01)     |
| Fat (%)           | 16.25 +/- 0.73   | 25.41 +/- 1.12   | 2.85X10⁻⁷ (<0.000001) |
| Lean (g)          | 20.78 +/- 0.74   | 15.78 +/- 0.53   | 8.18X10⁻⁶ (<0.00001) |
| Oxygen consumption (ml/g⁰.⁷⁵/hr) | 12.58 +/- 0.08 | 11.28 +/- 0.04 | 1.68X10⁻¹⁶ |

**Table 1:** Body composition and oxygen consumption measurements in 3 month old wild type control and TIS7 transgenic mice. Body composition was determined by dual-energy x-ray absorptiometry. N=13-15 control or transgenic mice per group. Oxygen consumption was quantified by indirect calorimetry as per (14). N=22 wild type, N=25 transgenic mice. Data are expressed as means +/- S.E.M. and p values were calculated by Student’s t test.
### Table 2: Analysis of intestinal nutrient transport gene expression

WT and TG mice on chow or high fat diets were sacrificed and intestines divided into duodenal, proximal (proximal jejunum), mid (distal jejunum) and distal (ileum) segments. Quantitative real time RT-PCR analyses were performed as per Materials and Methods. Results are expressed as the average fold change in gene expression (TG/WT) in all intestinal segments (intestine), or in proximal jejunum (high fat diet). The maximum fold change TG/WT, was found in the duodenum (n=3-4 WT and 4-6 TG, 3 month old males).

| Gene category          | Gene | Mean Fold Change TG/WT, intestine, chow diet | Maximum fold change TG/WT, chow diet | Mean fold change TG/WT, high fat diet |
|------------------------|------|---------------------------------------------|-------------------------------------|---------------------------------------|
| Lipid Metabolism       | MTP  | 1.2                                         | 1.9 in duodenum,                     | 1.25                                  |
|                        | DGAT1| 1.6                                         | 2.4 in duodenum                      | 1.2                                  |
|                        | DGAT2| 1.55                                       | 1.8 in duodenum                      | 1.3                                  |
| Nutrient absorption    | CRBPII| No change                                   | No change                           | ND                                   |
|                        | SGLT-1|                                             |                                     |                                       |
|                        | LFABP|                                             |                                     |                                       |
|                        | I-FABP|                                             |                                     |                                       |
| Serum levels | 1 MONTH Controls (WT) | 1 MONTH Transgenic | P value | 3 MONTH Controls (WT) | 3 MONTH Transgenic | P value |
|--------------|-----------------------|-------------------|---------|-----------------------|-------------------|---------|
| IGF-1, ng/ml | 592.40 +/- 10.23      | 402.04 +/- 41.42  | <0.05   | 476.8 +/- 7.46        | 354.65 +/- 14.02  | <0.001  |
| GH (ng/ml)   | 4.08 +/- 1.71         | 2.50 +/- 0.69     | NS      | 4.35 +/- 1.70         | 1.69 +/- 1.38     | NS      |
| Glucagon, pg/ml | 96.27 +/- 9.23   | 88.58 +/- 9.85    | NS      | 87.92 +/- 3.51        | 109.06 +/- 6.88   | <0.05   |
| Insulin, ng/ml | 0.62 +/- 0.29       | 0.55 +/- 0.17     | NS      | 0.32 +/- 0.12        | 0.32 +/- 0.1      | NS      |
| Leptin, ng/ml | 3.28 +/- 0.46        | 4.76 +/- 0.5      | NS      | 4.07 +/- 0.76        | 8.62 +/- 1.71     | =0.05   |
| Glucose      | 115.58 +/- 7.37      | 94.79 +/- 5.67    | <0.04   |

Table 3. Serum GH, IGF-1, glucagon, insulin, leptin and glucose levels in wild type (WT) and TIS7 transgenic (TG) mice. For GH, IGF-1, glucagon, insulin and leptin levels, serum was withdrawn from one-month old (n=8 WT, and n= 8 TG) or 3-month old (n=11 WT, n=13 TG) mice, strictly between 8:00 and 8:30 a.m. (NS, non-significant). For glucose levels, mice were fasted for four hours; n=13 WT, n=15 TG mice.
Figure 1

A

\[ \text{FabI}^{-96 \text{ to } -21} \quad \text{Tis7 cDNA} \quad \text{hGH}^{+3 \text{ to } +2450} \]

B

WT TG WT TG

Duodenum Jejunum

C

| Relative TIS7 mRNA levels | Duodenum | Proximal jejunum | Distal jejunum | Ileum |
|---------------------------|----------|------------------|----------------|------|
|                           | WT       | TG               | WT             | TG   |

D

WT TG WT TG

Duodenum Ileum

Downloaded from http://www.jbc.org/ by guest on March 24, 2020
Figure 3

A

B

Percentage weight change (female)

Percentage weight change (male)

Days on high fat diet

Days on high fat diet
Figure 5

A

B
Targeted intestinal over-expression of the immediate early gene TIS7 in transgenic mice increases triglyceride absorption and adiposity
Yuan Wang, Hristo Iordanov, Elzbieta A. Swietlicki, Lihua Wang, Christine Fritsch, Trey Coleman, Clay F. Semenkovich, Marc S. Levin and Deborah C. Rubin

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