SUPPLEMENTARY DATA

SUPPLEMENTARY MATERIAL

Caco-2 cell culture with GLP-1(1-37) and GLP-1(7-37) and ELISA. For co-culturing, 80% confluent monolayers of Caco-2 cells in 12-well plates were covered with 1 mL F12-K with 10% FBS and incubated at 37°C with 5% CO_2. 200 nM, 400 nM, 2 µM GLP-1 (1-37), or GLP-1(7-37) (Bachem, King of Prussia, PA), or 1X PBS were added separately into different wells. Following a 16 h incubation, an additional 1 mL F-12K with 10% FBS plus 200 nM, 400 nM, 2 µM GLP-1 (1-37), or GLP-1(7-37) was added to the cells, supplemented with 0.4% Glucose for an additional 2 h. The media was removed from the cells, supplemented with Leupeptin (10 ng/mL), 0.2 mM PMSF and aprotinin (10ng/mL), centrifuged (12,000 x rpm) (Effendorf 5804R, Westbury, NY), and kept briefly at 4°C prior to ELISA analysis for insulin expression. Insulin was measured using Human Insulin ELISA kit (Millipore, MA) according to the manufacturer's instructions. To assay for GLP-1(1-37) or GLP-1(7-37), the GLP-1 ELISA Kit (Millipore, MA) was used according to the manufacturer's instructions.

Rat Serum ELISA. Rat serum was obtained from all rats in the study and assayed using standard ELISA kits (Millipore, MA) for GLP-1(7-37) for the presence of GLP-1 (either GLP-1(1-37) or GLP-1(7-37) according to the manufacturer's instructions. Standard curves were made for GLP-1(7-37) vs. GLP-1(1-37) in order to obtain an estimate of the concentrations for each.

Detailed cloning for lactobacillus strains. A plasmid was constructed for expressing *glp-1(1-37)* under control of the SlpA promoter (SLPAP) to make pFD-GLPL. The sequence GLPL (SLPAP-USP45-LEISS-HIS-EK-GLP(1-37)) was made synthetically (IDT, Coralville, IA). This fragment was inserted via high fidelity PCR (New England) into pBluescript-LacII (In which the LacII gene from Lactobacillus was subcloned into pBluescript-KS) to make pBluescript-GLPL. The resultant vector contained the sequence LGLPL (LacII upstream-GLPL-LacII downstream). LGLPL was PCR amplified and ligated into pORI28 plasmid (NCK1609) to make pORI28-LGLPL in EC1000. Plasmid pORI28-LGLPL was transformed into L containing pTRK669 (NCK 1391), grown in MRS with chloramphenicol 5 µg/ml (cam5). Transformants were selected on MRS plates with 5 µg/ml erythromycin and 5 µg/ml chloramphenicol. Colonies are visible after 24-72 h incubation at 37°C grown anaerobically. Transformants were screened for integration in MRS plates with 2 µg/ml erythromycin and 2 µg/ml chloramphenicol at 37°C overnight. Cultures were transferred (1% inoculum) 2-3 times in MRS with 2 µg/ml erythromycin only at 42°C. Cells were plated on MRS plates with 2 µg/ml erythromycin to obtain isolated colonies. Plates were incubated at 37°C anaerobically for 48 h. Replica plating was carried out on MRS with 2 µg/ml erythromycin and MRS with 5 µg/ml chloramphenicol to screen for chloramphenicol-sensitive colonies. Integration was verified in selected chloramphenicol sensitive colonies by PCR.

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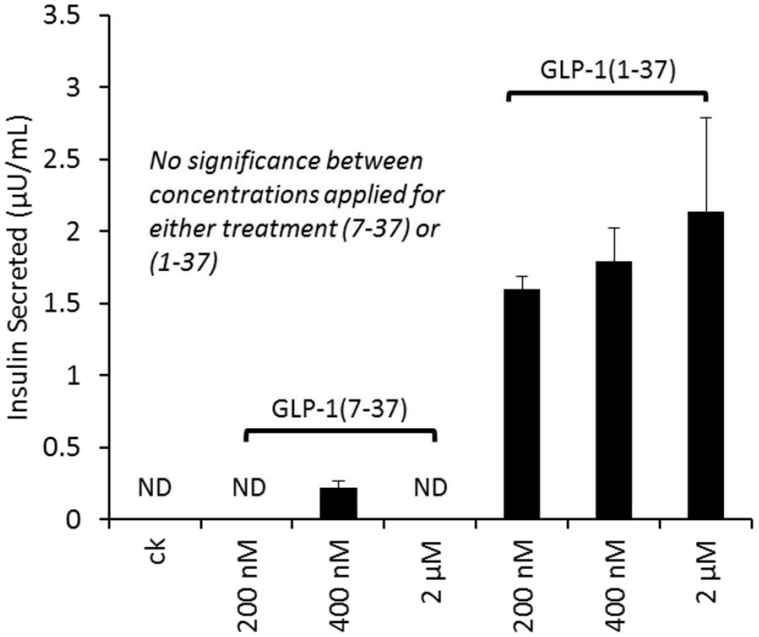
RT-PCR. Total RNA was isolated from the intestines of rats fed with LG or L and also from the pancreases and livers of healthy control rats and from IEC-6 cells using the RNAqueous ® kit (Life Technologies, NY). cDNA was synthesized by SuperScript ® III first-strand kit (Invitrogen, CA). The primers used for amplifying different genes as following.

mafA, (TCAACGACTTCGACCTGATG,GGGCAGAGTGATGATGGTG); *insulin 1* (AACCCTAAGTGACCAGCTACAATCATAG, GCAGGCTTGGGCTCCC); *insulin 2* (TAAGTGACCAGCTACAGTCGG, GCTCCCCACACCAGGTAG); *hnf6* (GGCCTCCATGAATAACCTCTATAC, TCTTCCATCTGCCCTGAATTAC); *ngn3* (CCATCCAAGTGTCCCAAGAG, GAGTCAGTGCCCAAATGTAGT); *sox9* (TTCATGAAGATGACCGACGA, GTCCAGTCGTAGCCCTTCAG); *foxA2* (CCTTCATCCGCACCCTATTT, GGGAATAGAGGCTCTGGTAGTA); *pdx-1* (CTCCGGTTCTGCTGCGTAT, CACAGCTCTACAAGGACCCG); *glp-1R* (GGTGGAAGGCGTGTATCTGT, AGATTAGCCTTCAGCTTGGCT); *insulin R* (AGAGGAACGACATTGCCCTG, TTTCGGGATGGCCTAGTGTC); *chrA* (TCAATCTCCAGGGGAGGACA, GGTCTGTGGAATCCCATGCT); *glucagon* (ATCAAAGACGCTGCCCTCAA, AACCAGTAGTAGTTGGCCGC); and *β-actin* (ACCCTAAGGCCAACCGTGAA, ACGCACGATTTCCCTCTCAG).

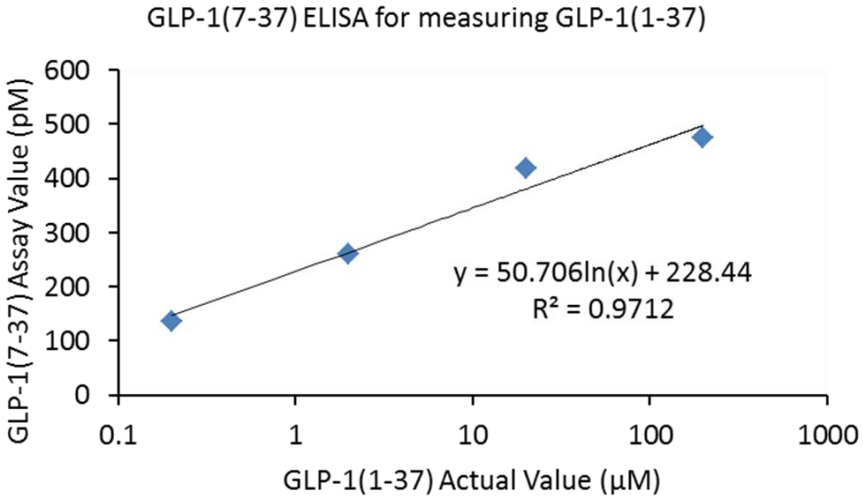
SUPPLEMENTARY RESULTS

Supplementary Figure 1. Bioassay for GLP-1(1-37) activity. GL-1(7-37) and GLP-1(1-37) were applied to human enteric carcinomas (Caco-2 cells) and the amount of secreted insulin was measured. The amount of GLP-1 applied is on the abscissa and the insulin secreted is on the ordinate. Values are averages and errors bars represent 1 st. dev. ND=none detected. Ck=blank control.

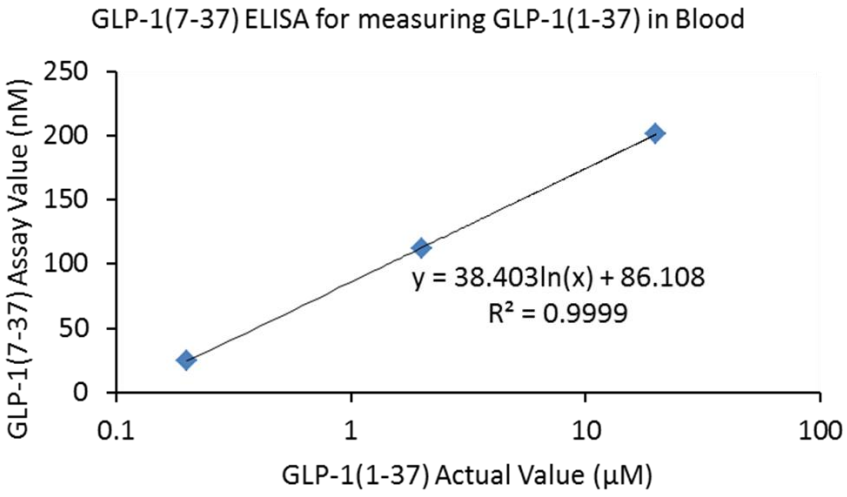


SUPPLEMENTARY DATA

Supplementary Figure 2. ELISA for GLP-1(1-37). Using a standard ELISA kit for GLP-1(7-37), an estimate for the amount of GLP-1(1-37) in both blood and assay buffer was determined. Shown here are **assay buffer** results. The amount of GLP-1(1-37) applied is on the abscissa and the estimate in GLP-1(7-37) equivalents is presented on the ordinate.



Supplementary Figure 3. ELISA for GLP-1(1-37). Using a standard ELISA kit for GLP-1(7-37), an estimate for the amount of GLP-1(1-37) in both blood and assay buffer was determined. Shown here are **blood** results. The amount of GLP-1(1-37) applied is on the abscissa and the estimate in GLP-1(7-37) equivalents is presented on the ordinate.



SUPPLEMENTARY DATA

Supplementary Figure 4. Blood levels of GLP-1. Using a standard ELISA kit for GLP-1(7-37), an estimate for the amount of GLP-1(1-37) in blood of treated and control rats was measured. The levels of GLP-1 from the assay revealed no difference between any of the samples. Conversion to GLP-1(1-37) values were below the lowest point on the standard curve and extrapolation revealed no difference in that compound as well (data not shown).