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Effects of Long- and Short-Chain Fatty Acids on the Release of Gastrointestinal Hormones using an ex Vivo Porcine Intestinal Tissue Model

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ABSTRACT: Gastrointestinal (GI) peptide hormones play an important role in short-term regulation of food intake and blood glucose levels. Modulating their release is of potential relevance for weight management and possibly diabetes. As currently available models are hard to extrapolate to the human situation, the use of porcine intestinal tissue, collected from slaughter pigs, was investigated for this purpose. Intestinal tissue disks showed a predicted regional release pattern of GI peptides. Various long-chain fatty acids differentially stimulated release of glucagon-like peptide 1 (GLP-1) (up to 500%) and glucagon-like peptide 2 (GLP-2) (up to 200%) from ileal tissue disks, but effects on peptide YY (PYY) did not reach significance. Short-chain fatty acids had no effects on the release of GLP-1, GLP-2, and PYY in either the ileum or colon. In conclusion, this porcine tissue model shows to be of advantageous use in a tiered approach to study the potential of satiety-inducing compounds to be selected for studies in humans.

KEYWORDS: *ex vivo porcine intestinal model, satiety, gut hormones, fatty acids*

INTRODUCTION

The sensing and control of nutrient levels are crucial for the maintenance of metabolic homeostasis, and a dysfunction of this homeostasis underlies many chronic metabolic diseases, including obesity, type 2 diabetes, and atherosclerosis. In the gastrointestinal (GI) tract, nutrients can trigger various signals, including stimulation of mechanoreceptors through gastric distension and the release of peptide hormones by intestinal cells, a process that has been extensively described.[1,2] These gut hormones act as short-term signals, most of them with anorexic effects: they promote satiation, which causes meal termination; or satiety, which postpones the initiation of a subsequent meal.[3] Besides these satiating effects, some GI hormones produce local effects on GI functions or they work as incretins, which means they stimulate insulin release and thereby lower blood glucose levels.[3] Hormones known to have an anorexic effect are cholecystokinin (CCK), which is mainly produced by I-cells in the proximal small intestine; and glucagon-like peptide (GLP)-1 and peptide YY (PYY), which are mainly produced by L-cells in the distal ileum and colon. GLP-1 is also a known incretin hormone, and also CCK and PYY have been shown to positively affect glucose homeostasis.[3,4] The effects on appetite and glucose homeostasis make these hormones an interesting target in the treatment and prevention of obesity and diabetes. The GI hormone GLP-2, also produced by enteroendocrine L-cells, does not induce satiety,[5] but has been shown to stimulate proliferation and facilitate repair of the intestinal epithelium in animal models for intestinal diseases[6] and is therefore implicated as a possible target for the prevention and treatment of different intestinal disorders.

The effects of nutrients and other compounds on the release of GI hormones have been extensively studied. Different macronutrients differentially stimulate the release of GI hormones,[1] probably because of different receptors on enteroendocrine and nearby intestinal cells. In general, lipids and protein hydrolysates seem to have the most potent effects.[2,7−10] Besides long-chain fatty acids (LCFAs) arising from ingested food, also short-chain fatty acids (SCFAs) are present in the intestinal lumen. SCFAs are the main products of bacterial fermentation of undigested food in the distal part of the intestine and colon.[11] Although SCFAs are known to interact with different GI receptors leading to physiological responses, their role in appetite regulation seems to be more on the longer term and particularly relevant for the colon. A few studies report that SCFAs trigger release of GI hormones, but the results are not consistent, which may be due to differences in the (cell) system used.[11−13] To study the effects of nutrients on the release of GI hormones in vitro, often enteroendocrine cell-based assays are used, such as the murine STC-1[14−16] and GLUTag[17] cell lines and/or the human NCI-H716 cell line.[18] The results of these assays using single cell type cell lines, however, are difficult to extrapolate to humans. Human in vivo studies of course represent the actual physiological situation best. The ability of different nutrients to stimulate the secretion of satiety peptides has been studied in humans by monitoring plasma levels after food intake. However, these studies are time- and cost-consuming and may be hampered by practical and ethical considerations. A system that resembles the human in vivo situation better than cell models might be the use of isolated intestinal tissue material. Intestinal tissue contains a mucus layer and all different cell types present in the intestine

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and, therefore, simulates the human intestinal physiology in a better way than cell cultures, but with a higher feasibility than human in vivo studies. The gastrointestinal system of pigs has high anatomical and physiological similarities to the human digestive system and can therefore be used to simulate the human GI tract.[19,20]

The aim of this study was to develop a new ex vivo porcine intestinal tissue model to screen potential satiety-inducing compounds. To determine the suitability of the model, the presence of GI hormones along the gastrointestinal tract, their release upon stimulation with known stimulants, and tissue viability were studied. In addition, the effects of long- and short-chain fatty acids on hormone release from different parts of the GI tract were determined using this ex vivo porcine intestinal tissue model.

MATERIALS AND METHODS

Chemicals. The long-chain fatty acids palmitic acid (C16:0) and stearic acid (C18:0) and the short-chain fatty acids acetic acid (C2:0), proprionic acid (C3:0), butyric acid (C4:0), valeric (or pentanoic) acid (C5:0), and caproic (or hexanoic) acid (C6:0) were all from Sigma, Zwijndrecht, The Netherlands. Oleic acid (C18:1) was obtained from Mundipharma, Cambridge, UK. Arachidonic acid (C20:4 *n*-6) was from Fluka, Zwijndrecht, The Netherlands. Eicosapentaenoic acid (C20:5 *n*-3) and docosahexaenoic acid (C22:6 *n*-3) were both from Cayman Chemicals, Ann Arbor, MI, USA. Ethanol 96% was supplied by Merck, Darmstadt, Germany. Casein hydrolysate was obtained from DMV International, Veghel, The Netherlands. H_2O_2 was from Dako, Carpintera, CA, USA. All other chemicals were of analytical grade and obtained form Sigma.

Collection of Porcine Intestinal Tissues. Intestinal tissues were obtained from pigs (*Sus scrofa domesticus*) killed for meat production at a local slaughterhouse. Pigs were commercial breeds (Toppig 20 × Pietrain), around 6 months old, weighing approximately 90 kg at slaughter, and had been fasted for approximately 12 h prior to slaughter. Within 15 min after slaughter, intestines were excised and segments of different anatomical regions were stored in ice-cold oxygenated (95% O_2, 5% CO_2), Krebs' Ringer bicarbonate (KRB) buffer containing 25 mM HEPES and adjusted to pH 7.4, further referred to as KRB/HEPES. For analysis of tissue GI hormone levels, samples were collected at seven different positions along the GI tract. These were labeled as duodenum (sample collected 10 cm after pylorus), proximal jejunum, mid jejunum, distal jejunum (taken at 1.5, 5, and 10 m after the pylorus, respectively), proximal and distal ileum (sampled at 5 and 1.5 m before the ileocecal junction), and colon (collected approximately halfway along the colon). For ex vivo stimulation experiments intestinal tissue was collected from the proximal jejunum, distal ileum, and colon. Tissues were stored in ice-cold oxygenated KRB/HEPES, transported to the laboratory, and immediately used for ex vivo stimulation (time between excision and studies was approximately 45−60 min).

Preparation of Dose Solutions. Casein hydrolysate was used as a positive control for hormone release from the tissues. The protein hydrolysate powder was diluted in warm KRB/HEPES (37 °C) shortly before each experiment. Concentrations used were between 1 mg/mL (0.1%) and 40 mg/mL (4%; w/v) casein hydrolysate. Long-chain fatty acids used were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), arachidonic acid (C20:4 *n*-6), eicosapentaenoic acid (C20:5 *n*-3), and docosahexaenoic acid (C22:6 *n*-3). The long-chain fatty acids were first dissolved in ethanol (96%) and further diluted in KRB/HEPES to concentrations of 0.5 mM and lower prior to stimulation experiments. The final ethanol concentration was ≤1.0% in all dose solutions. The short-chain fatty acids used in the experiment were acetic acid (C2:0), proprionic acid (C3:0), butyric acid (C4:0), valeric (or pentanoic) acid (C5:0), and caproic (or hexanoic) acid (C6:0). The water-soluble short-chain fatty acids were dissolved and diluted with KRB/HEPES to concentrations of 5 mM and lower. Before exposure to the intestinal tissue, the pH of the dosing solutons was measured and adjusted to 7.4.

Ex Vivo Stimulation of GI Hormone Release and Viability Studies. Upon arrival at the laboratory, the intestine was rinsed with KRB/HEPES and cut open in a longitudinal direction. With the basolateral side upward, the outer muscle layers were carefully stripped off. The mucosal tissue was placed on gauze (pores = 250 μm, Sefar Nitex 03-250/50, Sefar, Heiden, Switzerland) with the apical side upward, and circles with a diameter of 8 mm (0.50 cm^2) were punched out using a Biopsy punch (Stiefel, Offenbach am Main, Germany). The intestinal segments were transferred to a 24-well plate kept on ice and filled with 500 μL KRB/HEPES per well. To study the secretion of GLP-1, CCK, and PYY from intestinal tissue, the 24-well plate containing the intestinal segments was brought to room temperature. Studies were initiated by replacing the buffer solution with 500 μL of prewarmed KRB/HEPES dose solutions. The tissues were incubated for 1 h at 37 °C in a humidified incubator at 5% CO_2. After incubation, incubation media were centrifuged for 5 min at 10000*g*, and samples of supernatant were collected for further analysis of lactate dehydrogenase (LDH) and different GI hormones. Samples for GI hormones analysis and intestinal segments were stored below −70 °C upon analysis.

To check the viability of intestinal tissue due to the possible presence of active proteases, leakage of intracellular LDH was determined. In addition, macroscopic and microscopic checks were performed after a hematoxylin−eosine histology staining. Tissue hormone levels and LDH were determined in 0.5 cm^2 tissue samples, which were homogenized in ice-cold KRB/HEPES with a Potter-Elvehjem-type Teflon pestle tissue grinder (Braun, Melsungen, Germany) for 5 min at 200 rpm.

Determination of CCK Levels. CCK levels were determined using a EURIA-CCK radioimmunoassay (RIA) kit (Euro-diagnostica, Malmö, Sweden) according to the manufacturer's instructions. An identical sequence of CCK-8 has been found for most mammals, among them pigs and human. The kit measures CCK-8 sulfate (CCK (26-33)), with a detection limit of 0.3 pM. The intra-assay variation ranges from 2.0 to 5.5% and the interassay variation from 4.1 to 13.7%. Cross-reaction with gastrin is 0.5%. Radioactivity of [^{125}I]-CCK-8 was measured by liquid scintillation counting using a gamma counter (EG&G, Breda, The Netherlands).

Determination of GLP-1 Levels. GLP-1 levels were determined using a sandwich enzyme-linked immunoabsorbent assay (ELISA). The primary antibody to GLP-1 [5 μg/mL mouse monoclonal (8G9) in PBS, Abcam, Cambridge, UK] was coated on a flat-bottom 96-well plate (Nunc, Roskilde, Denmark) during 4 h at 37 °C. This primary antibody is specific for the amidated C terminus of the peptide and reacts with GLP-1 (1−36) and GLP-1 (7−36), but not with GLP-1 (7−37). After blocking the plate using a PBS buffer containing 0.1% v/v Tween 20 and 4% w/v BSA, the plate was washed four times with PBS buffer containing 0.1% v/v Tween 20. A standard curve with GLP-1 concentrations ranging from 0 to 1000 pM was prepared in KRB/HEPES, and samples were diluted if needed. Samples and standards were added to the microtiter plate and incubated with the primary antibody for 2 h at room temperature. Subsequently, the plate was washed four times, and the wells were incubated with a secondary biotinylated antibody to GLP-1 [2 μg/mL; mouse monoclonal (HYB 147-08); Biotrend, Cologne, Germany] for 2 h. After another washing step, samples were incubated with streptavidin−horseradish peroxidase (1:2000, Pharmingen, Heidelberg, Germany), followed by an incubation with TMB solution (containing 3,3′,5,5′-tetramethylbenzidine and H_2O_2). The reaction was stopped by adding H_2SO_4 (1 M), and absorbance of the yellow end product was measured at 450 nm on a microtiter plate spectrophotometer (Benchmark plus, Bio-Rad laboratories, USA). The concentration in the samples was determined by interpolation to the concentrations of the standard solutions. The range of the assay was approximately 50−1000 pM. The intra-assay variation coefficient ranged from 1 to 9% and the interassay variation coefficient from 18 to 35%.

Determination of GLP-2, PYY, and LDH. GLP-2 levels were determined using a competitive human GLP-2 (1-34) ELISA kit from

Phoenix Pharmaceuticals, Inc. (Belmont, CA, USA), according to the manufacturer's instructions. There is a 92% homology of GLP-2 protein between human and pigs. This kit has a lower detection limit of 0.16 ng/mL (0.04 pmol/mL) and an intra-assay variation from 5 to 10%. Cross reaction with GLP-1 is 6%.

PYY levels were measured using a PYY ELISA kit for total PYY (Bachem, Peninsula Laboratories, San Carlos, CA, USA) according to the manufacturer's instruction. This kit can measure porcine PYY, which is identical to human PYY.[21] The lower detection limit was 20 pg/mL (4.6 pmol/mL).

LDH, a stable cytosolic enzyme that is released upon cell damage, was measured to determine LDH leakage. LDH activity was determined using a Roche reagent kit (Roche Diagnostics, USA) on a Hitachi 911 centrifugal analyzer.

Statistical Analysis. Data are expressed as absolute amounts or as percentage of control. Data are presented as the mean and standard deviations (SD). Statistical differences between group means were determined by Student's two-tailed t test, for unpaired samples. Differences were considered to be statistically significant at $P < 0.05$. P values were assigned at three different levels: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. The effect of LCFAs and SCFAs on the release of satiety hormones was analyzed with one-way analysis of variance (ANOVA) followed by Dunnett's test: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. The number of samples (n) refers to the number of intestinal tissue pieces used.

RESULTS

Levels of GI Hormones in Porcine Intestinal Tissue. A clear pattern of tissue levels of CCK, GLP-1, GLP-2, and PYY along the porcine intestinal tract was observed (Figure 1).

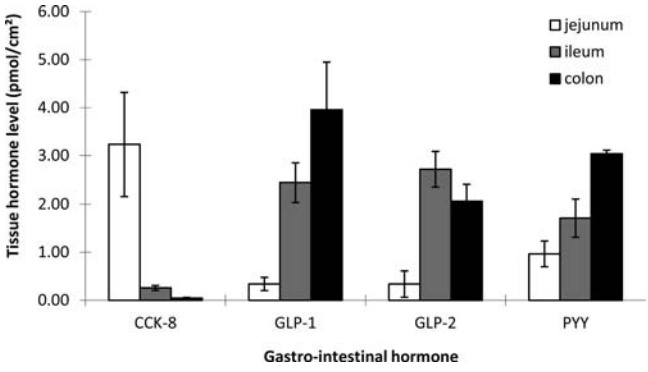

Figure 1. Tissue hormone levels of cholecystokinin (CCK), glucagon-like peptides 1 and 2 (GLP-1 and GLP-2), and peptide YY (PYY) in porcine jejunum, ileum, and colon tissues. Data are expressed as the mean ± SD in pmol/cm^2 tissue (n = 3 for CCK and PYY; n = 4 for GLP-2 and GLP-1).

CCK, known to be predominately released from the I cells in the proximal intestine, was present at levels of 3.84 ± 1.08 and 0.26 ± 0.05 pmol/cm^2 in jejunal and ileal tissues, respectively. In colonic tissue, hardly any CCK could be detected. Tissue levels of GLP-1, GLP-2, and PYY, hormones known to be predominately released from the L cells in the ileum and colon, increased along the porcine GI tract. For GLP-1, the tissue levels measured were 0.34 ± 0.14 pmol/cm^2 in the jejunum, 2.44 ± 0.41 pmol/cm^2 in the ileum, and 3.96 ± 0.99 pmol/cm^2 in the colon. For GLP-2, the tissue levels in jejunum and ileum were comparable to GLP-1 levels at 0.34 ± 0.27 and 2.72 ± 0.38 pmol/cm^2, respectively. GLP-2 levels in the colon were slightly lower as compared to GLP-1 at 2.06 ± 0.36 pmol/cm^2. PYY levels measured were 0.97 ± 0.27, 1.71 ± 0.40, and 3.04 ± 0.08 pmol/cm^2 in jejunal, ileal, and colonic tissues, respectively.

To study the distribution of GLP-1 along the porcine intestinal tract in more detail, tissue GLP-1 levels were determined in seven different tissue segments (Figure 2).

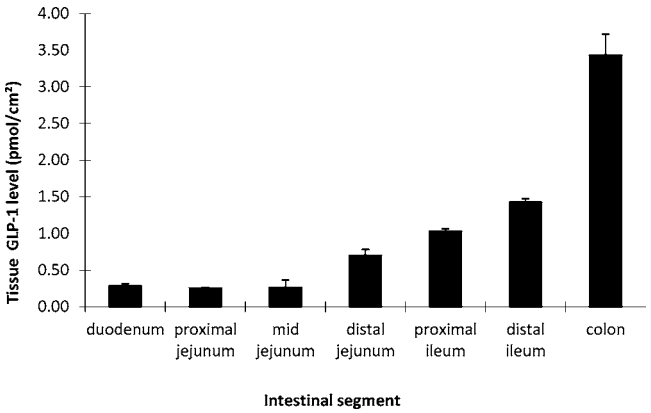

Figure 2. Tissue hormone levels of glucagon-like peptide 1 in seven consecutive gastrointestinal segments of the porcine gastrointestinal tract. Data are expressed as the mean ± SD in pmol/cm^2 tissue (n = 4).

GLP-1 levels increased along the GI tract from 0.28 ± 0.03 pmol/cm^2 in the duodenum and comparable amounts in the proximal and mid jejunum. Further down the GI tract, GLP-1 levels increased to 0.75 ± 0.12 pmol/cm^2 in the distal jejunum, to 1.03 ± 0.03 pmol/cm^2 in the proximal ileum, and to 1.43 ± 0.05 pmol/cm^2 in the distal ileum. The highest concentration of GLP-1 was measured in the colon, which contained 3.44 ± 0.29 pmol/cm^2.

Viability of ex Vivo Intestinal Tissue and Basal Hormone Release. To determine the integrity of the ex vivo intestinal tissues, the viability was first monitored by histological examination (data not shown) and further in all experiments by measuring the leakage of intracellular LDH into the medium. Total intracellular LDH levels measured were 4.27 ± 0.24, 4.35 ± 0.15, and 2.71 ± 0.22 U/cm^2 in the jejunum, ileum, and colon, respectively, indicating the reduction in intestinal cells per square centimeter due to the smaller reduced size of the villi in the colon. In all experiments, after 1 h of incubation, LDH leakage from the ileum and colon remained below 10% of the intracellular LDH levels, whereas LDH leakage from jejunal tissue was significantly higher with levels up to 20% of total LDH. In contrast to these differences in LDH leakage, no difference in basal release of hormones could be detected among the jejunum, ileum and colon after exposure to buffer solely as this was below 10% for all tissues.

Release of GI Hormones upon Exposure to Casein Hydrolysate. Casein hydrolysate was used as a positive control for the release of GLP-1, GLP-2, and PYY from the porcine intestinal tissues. As expected, exposure of jejunal tissue to casein hydrolysate (0.1, 0.5, 1, 2, and 4% w/v) resulted in hardly any release of GLP-1, GLP-2, and PYY, whereas exposure of ileal and colonic tissues resulted in a high release of these hormones in a dose−response manner up to 1% casein hydrolysate (data not shown). Casein hydroysate (1.0%) was further used as positive control in all experiments. To determine the optimal incubation time, hormone release and LDH leakage were measured up to 2.5 h of exposure to casein hydrolysate (Figure 3). Hormone release increased with longer incubation time, but tissue damage also increased. An

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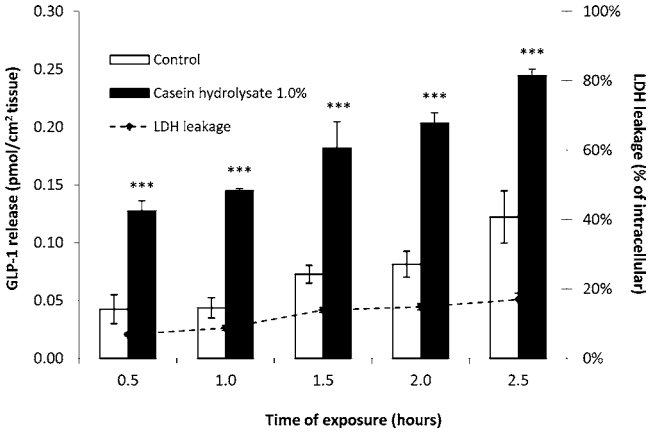

Figure 3. Release of GLP-1 from ileum tissue after incubation with control buffer or casein hydrolysate (1%), in comparison with LDH leakage (dashed line). Data are expressed as the mean ± SD in pmol/cm^2 tissue (n = 3). Total LDH was 4.35 ± 0.15 units/cm^2. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$, significant difference (Student t test) from corresponding control.

incubation period of 1 h was chosen for further experiments. Some interanimal variation in the response of ileal tissue to 1% casein hydrolysate was observed for the release of GLP-1 (425 ± 79%; n = 8 pigs), PYY (524 ± 98%, n =5 pigs), and GLP-2 (438 ± 132%, n = 3 pigs).

Effects of Long-Chain Fatty Acids on the Release of GI Hormones. LCFAs are known stimulants of the release of several intestinal hormones and may therefore be regarded as another positive control. All six LCFAs tested, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), arachidonic acid (C20:4 n-6), eicosapentaenoic acid (C20:5 n-3), and docosahexaenoic acid (C22:6 n-3), triggered the release of GLP-1 and, to a lesser extent, GLP-2 from ileum tissue in the ex vivo model (Figure 4). The six LCFAs approximately doubled GLP-2 and PYY concentrations, but for PYY this difference did not reach significance compared to control. With regard to stimulation of GLP-1 release, the fatty acids differed in their effects. Palmitic (16:0) and stearic acid (C18:0) stimulated the release of GLP-1 to approximately 250%. Fatty acids with longer C chains and a higher degree of unsaturation produced a more pronounced stimulation of GLP-1 release, to approximately 500% as compared to control buffer solution.

Effects of Short-Chain Fatty Acids on Release of GI Hormones. In addition to the LCFAs, effects of SCFAs on the release of GI hormones were studied. Intestinal tissue was incubated with SCFAs with chain lengths of two to six carbon atoms. For these experiments only ileum and colon tissues were included in the study, because, in vivo, SCFAs are produced by microbiota in the distal parts of the GI tract. No release of GLP-1, GLP-2, or PYY could be detected after exposure of the tissue to 5 mM SCFAs, from either ileum (Figure 5) or colon tissue (Figure 6). Also, lower concentrations of SCFAs (0.5 mM) did not result in release of GLP-1, GLP-2, and/or PYY from ileal and colon tissues (data not shown).

DISCUSSION

The in vitro release of satiety hormones is often studied either using rodent intestinal tissue or entero-endocrine cell lines such as the STC-1 cell line. The most commonly used human cell line, NCI-H716, originally derived from a colorectal carcinoma, possesses L-cell-like properties. It secretes GLP-1 and has been reported to express a number of nutrient receptors.[22] Apart from lacking a normal cellular environment, this model requires the use of (expensive) attachment matrices as the cells normally grow in suspension while forming aggregates.[22] In addition, human biopsy tissue mounted in Ussing chambers can be used.[23] However, healthy human intestinal tissue is difficult to obtain. The model described here using ex vivo porcine intestinal tissue is relatively cheap and simple. Provided the presence of a small-scale slaughter facility, there is hardly any limitation of material supply.

The distribution of tissue levels of several hormones along the GI tract was as described before in pigs and humans, with high CCK levels in the jejunum and low CCK levels in the ileum and colon,[23,24] whereas GLP-1, GLP-2, and PYY levels showed an opposite distribution pattern with low levels in the jejunum and high levels in the ileum and colon.[21,25,26] Interestingly, when the distribution pattern of GLP-1 and

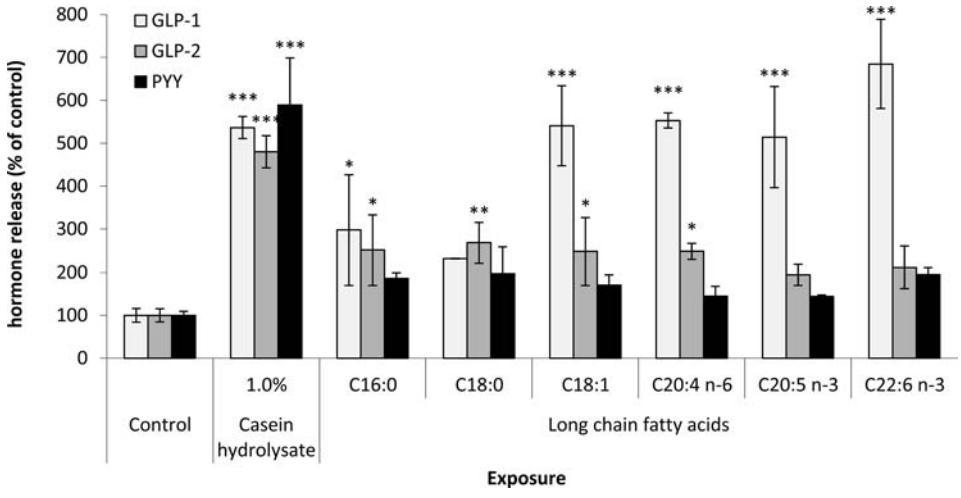

Figure 4. Hormone release from ileum tissue after incubation with different long-chain fatty acids (100 μM), expressed as percentage of control. Data are the mean ± SD in percentage of control (n = 3). Basal release, set at 100%, was 0.21 ± 0.03 pmol/cm^2 for GLP-1, 0.22 ± 0.03 pmol/cm^2 for GLP-2, and 0.11 ± 0.01 pmol/cm^2 for PYY. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$, significant difference (ANOVA + Dunnett's test) from corresponding control.

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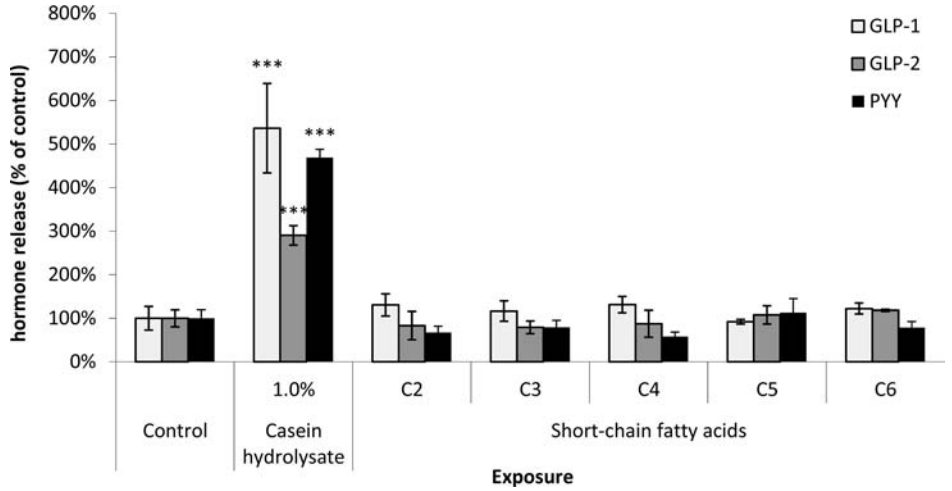

Figure 5. Hormone release from ileum tissue after exposure to different short-chain fatty acids (5 mM). Data are the mean ± SD in percentage of control (n = 3). Basal release from ileum was 0.06 ± 0.01 pmol/cm^2 for GLP-1, 0.29 ± 0.01 pmol/cm^2 for GLP-2, and 0.44 ± 0.05 pmol/cm^2 for PYY. ***, P < 0.001, significant difference (ANOVA + Dunnett's test) from corresponding control.

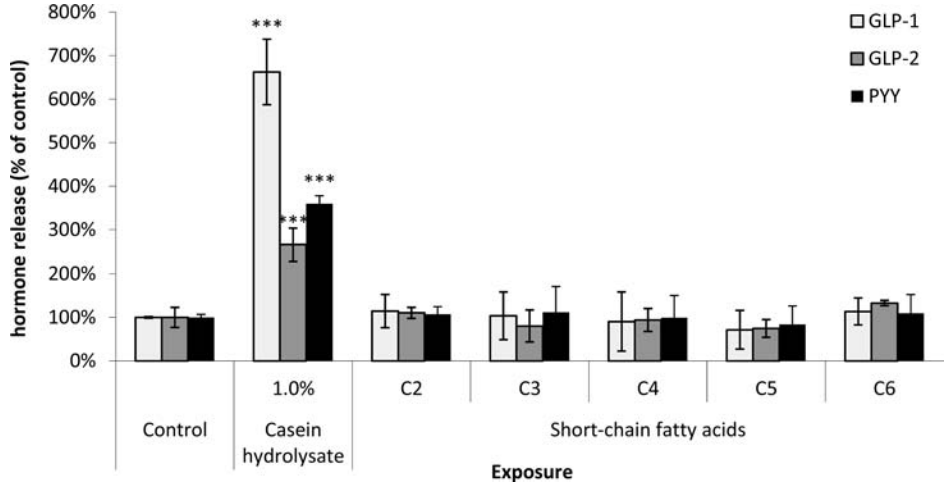

Figure 6. Hormone release from colon tissue after exposure to different short-chain fatty acids (5 mM). Data are the mean ± SD in percentage of control (n = 3). Basal release from colon was 0.04 ± 0.001 pmol/cm^2 for GLP-1, 0.25 ± 0.06 pmol/cm^2 for GLP-2, and 0.60 ± 0.12 pmol/cm^2 for PYY. ***, P < 0.001, significant difference (ANOVA + Dunnett's test) from corresponding control.

GLP-2 along the GI tract was studied in more detail, a clear increase of GLP-1 levels was observed from proximal to distal, which was different from the distribution of GLP-2. Differences in absolute levels of GLP-1 and GLP-2 were observed in colon tissue and might be explained by differences in post-translational processing of their common precursor.[27] Only limited literature data are available for GLP-2 levels in the GI tract, but both GLP-1 and GLP-2 have been found to be present and secreted separately from porcine ileal tissue in a previous study.[28] Although interindividual differences in absolute tissue hormone levels were observed between different animals, the distribution pattern of hormones over the GI tract remained the same.

The low leakage of the viability marker LDH in addition to the low basal release of satiety hormones from ileum and colon tissues indicated that these tissues remained viable during the incubations. Experiments with jejunal tissue indicated a somewhat higher leakage of LDH, which corresponded with the histological observations. These differences in LDH leakage from the various tissues might be attributed to a higher rate of epithelial cell renewal in the proximal small intestine and therefore higher shredding of epithelial cells.[29] This could make the use of jejunal tissue in the ex vivo porcine model less suitable.

Leakage of LDH did not differ when tissues were incubated with different concentrations of casein hydrolysate or control solution only. Exposure of tissues to the well-known GI hormone stimulant, casein hydrolysate, at levels up to concentrations of 4.0%, did not affect tissue viability. Casein hydrolysate could therefore be used as a positive control in this model. Casein hydrolysate clearly stimulated release of GLP-1, GLP-2, and PYY from the ileum and colon in a dose-dependent manner, indicating that the model could be used to screen the potency of new compounds to stimulate GI hormone release. It should be noted that in the present model the test compounds may affect the intestinal tissue via both the apical and basolateral side. Some interanimal variation was observed for the absolute amounts of hormone release, which will be associated with the random sampling of pigs from the slaughter facility. Differences in feeding status, transport stress, and genetic factors are likely to play a role in this.

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Next, we studied the effects of LCFAs, known stimulants of GI hormone release, in this model. All LCFAs tested stimulated release of GLP-1 and, to a lesser extent, GLP-2 and PYY from porcine ileal tissue in accordance with the literature. Several fatty acid sensing G-protein coupled receptors (GPRs) that could be responsible for the effects of fatty acids on GI hormone release have been identified. GPR120 is expressed in the intestine and is activated by long-chain fatty acids in the gut, and activation has been shown to stimulate release of GLP-1[30] and CCK[31] in cell lines and mice in vivo. Another receptor, GPR40, has also been shown to be activated by long- and medium-chain fatty acids and to mediate release of GLP-1 in mice.[32] Recently, GPR119 has been shown to be activated by the fatty acid derivative oleoylethanolamine (OEA) and to mediate GLP-1 release.[33]

For GLP-1, differences were observed between saturated and unsaturated fatty acids, with markedly higher release when exposed to the unsaturated FAs. Also, in previous studies, unsaturated fatty acids have been shown to be more potent stimulators of GLP-1 release than saturated fatty acids, both in vitro and in studies with human volunteers.[17,34−36] Also, differences between medium- and long-chain fatty acids have been observed in previous studies.[17] These differences in evoking GLP-1 release between saturated and unsaturated FAs and between FAs with long or shorter chains could be explained by the different receptors that might be involved in FA-triggered GLP-1 release. Exposure to LCFAs also stimulated the release of GLP-2 from ileal tissue in our ex vivo system. The mechanism behind GLP-2 release is not well studied yet. In human studies, intake of a meal rich in fat and intraduodenal infusion of dodecanoic acid (C12:0) resulted in increased plasma levels of GLP-2.[37] The present study provides further evidence for the stimulating effects of some LCFAs on GLP-2 release. Contrary to the stimulation of GLP-1 release, all LCFAs tested enhanced GLP-2 release to the same extent in our model, although not significantly differently for all LCFAs. GLP-1 and GLP-2 are derived from the same propeptide, which suggests that additional mechanisms play a role in the processing, storage, or release of these peptides. As GLP-2 might be a target in the prevention or treatment of certain intestinal diseases,[7] our model could be useful to identify the chemosensory mechanisms involved in the release of GLP-2. The increase observed for PYY release in response to LCFAs in our study was small and not significant, which is in contrast to results from previous in vivo and in vitro studies.[8,16,38] Not much is known yet about the mechanisms by which LCFAs stimulate PYY release. In the current study, all LCFAs tested were found to slightly enhance PYY release, and there were no differences between saturated and unsaturated LCFAs.

In contrast to LCFAs, many fewer data are available on whether SCFAs (C2−C6) are able to stimulate GI hormone release. SCFAs are the major end products of bacterial fermentation of undigested food in the large intestine and are present in large concentrations in the colon and portal blood.[11] The different SCFAs are produced in different amounts depending on the composition of the microbiota and the diet. Previous studies have indicated that SCFAs can stimulate PYY release in vivo. Colonic and ileal infusion of SCFAs in rats and pigs has been shown to stimulate PYY release,[12,13] but not GLP-1 release.[12,13] In a recent human study, rectal infusion of acetic acid resulted in increased PYY release.[14] The free fatty acid receptors FFA2 and FFA3 (previously GPR43 and GPR41), which are present in multiple tissues including the intestinal tract, have been identified as SCFA receptors[39,40] and are expressed in enteroendocrine cells.[11] Besides effects of SCFAs directly, some studies have shown a relationship between dietary fiber intake—the substrate for SCFA production by microbiota—and GI hormone release. Treatment of rats with a diet rich in fiber resulted in an increase in GLP-1 and PYY levels.[41] Also, in dogs fed a high fermentable fiber diet, increased plasma GLP-1 levels were measured.[42] This last effect was associated with an increase in SCFAs,[42] indicating that fiber might increase GLP-1 release through increased SCFA production.

In the present study, however, no stimulation of GLP-1, GLP-2, or PYY release was observed after exposure of ileal and colonic tissue to SCFAs in different concentrations. This does not correspond with previous studies mentioned above, which found stimulation of PYY release. A difference with the current study was that all previous studies that found an effect were in vivo studies, indicating that additional mechanisms might have been responsible for the effects in these studies. For the effects of fiber, colonic distension could play a role in the stimulation of hormone release. Ingestion of fiber has been shown to increase fecal bulk and stool weight. Rectal infusion of saline solution elicited only PYY release in pigs, indicating that mechanical distension of the rectum stimulates PYY secretion.[12] This could also explain the difference between the effects of SCFAs and fiber on GLP-1 release in previous studies. Another explanation for this discrepancy could be that SCFAs exert their effects more distantly from the epithelial surface. Rectally infused SCFAs have been shown to be taken up in the circulation quickly.[43] Not only rectal infusion, but also intravenous infusion of acetate, has been shown to result in an increase in plasma PYY levels.[14] Besides effects on hormone release through mechanical distension or after uptake in blood, the differences in the effects of SCFAs in in vivo studies and the ex vivo model could also be explained by effects of SCFAs on gene expression instead of, or in addition to, direct effects on hormone release. Parenteral administration of SCFAs, for example, has been shown to increase proglucagon mRNA levels in rats.[44]

The porcine GI tract can provide a good model for the human GI tract because of anatomical and physiological similarities.[20] Both the distribution and absolute amounts of GI hormones have been found to be similar in intestinal tissue from pigs and humans,[21,26] which is clearly not the case for rat tissue, which showed different responses in GI hormone release as compared to human intestinal tissue.[23] The present ex vivo intestinal porcine tissue model can be used in a tiered approach to screen potential satiety-inducing compounds. Further studies are needed to characterize the model in terms of the presence of different receptors and to investigate whether this porcine intestinal tissue model can be used to study the intestinal barrier function in a broader sense.

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Notes

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■ ABBREVIATIONS USED

AA, arachidonic acid; CCK, cholecystokinin; DHA, docosahexaenoic acid; ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; FA, fatty acid; GI, gastrointestinal; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2; GPR, G-protein coupled receptor; KRB, Krebs' Ringer bicarbonate; LCFA, long-chain fatty acid; LDH, lactate dehydrogenase; OEA, oleoyl ethanolamine; PA, palmitic acid; PYY, peptide YY; SA, stearic acid; SCFA, short-chain fatty acid.

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