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Deliverable No. 3.3

Report on kinetics and gut hormone release after SCFA delivery

Grant Agreement no.: 101060247

Project Title: Innovative pulse and cereal-based food fermentations for human health and sustainable diets

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Responsible partner: KU Leuven

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Deliverable number	**D3.3**
Deliverable title	**Report on kinetics and gut hormone release after SCFA delivery**
Type[1]	R
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Work package number	3
Work package leader	Kristin Verbeke
Author(s)	Riet Rosseel, Kristin Verbeke
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Checklist	**Y (Yes), N (No)**
1) The deliverable has been sent to all relevant partners for review (i.e. in the same task/WP):	NA
2) The deliverable has been endorsed by the WP lead and co-lead:	Y
3) Input from other WPs has been sought, if applicable: ***Please specify:***	NA

[1] **Type**: Use one of the following codes (in consistence with the Description of the Action):
R: Document, report (excluding the periodic and final reports)
DEM: Demonstrator, pilot, prototype, plan designs
DEC: Websites, patents filing, press & media actions, videos, etc.

[2] **Dissemination level**: Use one of the following codes (in consistence with the Description of the Action)
PU: Public, fully open, e.g. web
SEN: Sensitive, limited under conditions of the Grant Agreement

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Publishable Summary

Please insert max ½ page on the progress made that led to the compilation of this deliverable. The text inserted here should be non-confidential as it might be used for project communication.

Short-chain fatty acids (SCFA) are microbial-produced metabolites that potentially mediate various metabolic health benefits. They are predominantly produced in the human colon through fibre fermentation and absorbed by colonocytes. In contrast, fermented foods contain SCFA, which are absorbed in the small intestine. Unlike colonocytes, small intestinal epithelial cells do not utilise SCFA as an energy source. Therefore, we hypothesised that a greater proportion of SCFA would reach the systemic circulation following small intestinal SCFA administration. In contrast, SCFA stimulate enteroendocrine L-cells in the gut, triggering the release of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which regulate appetite and glucose homeostasis. As L-cells are more abundant in the colon, we hypothesised that gut hormone release would be more pronounced following colonic SCFA administration. These hypotheses were evaluated in two studies with healthy volunteers using targeted delivery capsules that administered SCFA specifically in the small intestine and colon. Systemic availability was evaluated in the first study using capsules filled with ^{13}C-labeled SCFA. Gut hormone release was investigated in the second study using capsules containing unlabeled SCFA, with an additional placebo-controlled study visit to account for the hormone release induced by the meals consumed during the visit. Systemic availability was higher for propionate after small intestinal administration compared to colonic administration. However, no significant differences were observed for acetate and butyrate. GLP-1 and PYY release increased with SCFA administration in both the small intestine and the colon. However, only PYY release was significantly higher with colonic compared to small intestinal delivery. While the gut hormone release did not affect the glucose homeostasis, SCFA administration did impact appetite. Small intestinal SCFA administration reduced hunger, whereas colonic SCFA administration increased satiety and reduced desire-to-eat compared to placebo. In addition, small intestinal delivery increased fullness and satiety and reduced desire-to-eat compared to colonic SCFA delivery. Systemic SCFA concentrations were higher following small intestinal administration compared to colonic delivery. Although this data could not be used to calculate the systemic availability, this observation suggests that the hypothesis of the systemic availability may still be valid, also for acetate and butyrate.

1. Introduction

Please insert a short introduction based on the objectives and a short description of the corresponding WP according to Annex I (chapter "Objectives" and "Description of Work").

Deliverable 3.3 focuses on short-chain fatty acids (SCFA) as health-supporting metabolites. SCFA, primarily acetate, propionate and butyrate, are microbial-produced metabolites and potentially mediate various metabolic health benefits. To better understand their mechanisms of action, we investigated their kinetics and their impact on gut hormone release. SCFA are primarily produced in the human large intestine through the fermentation of dietary fibres and are absorbed by colonocytes. In contrast, fermented foods contain SCFA that are absorbed in the small intestine. Since small intestinal epithelial cells do not use SCFA as an energy source, unlike colonocytes, we hypothesised that a higher fraction of SCFA might enter the systemic circulation when absorbed in the small intestine, potentially inducing more pronounced effects on peripheral tissues [1]. In contrast, SCFA also stimulate enteroendocrine L-cells in the gut to release hormones such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which are involved in appetite regulation and glucose homeostasis. Since enteroendocrine L-cells are predominantly found in the large intestine, we hypothesised that the hormone release and its subsequent effects would be less pronounced after small intestinal compared to colonic administration [2, 3]. These two hypotheses were evaluated in two separate human intervention studies to be able to choose an optimal design for each outcome parameter. The primary outcome of study 1 was the systemic availability of SCFA, while study 2 focused on the gut hormone release and its subsequent impact on subjective appetite and glucose regulation.

2. Description of Activities

- Describe the work that you did in all clarity and detail necessary; there is no page limit.
- Provide figures with figure legends, describe material and methods
- Boldface important parts

2.1 Study 1: kinetics of SCFA

Thirteen healthy volunteers (3 male/9 female; age: 28 ± 8 years; BMI: 22 ± 2 kg/m²) visited the study centre for two study visits of each 12.5 hours, with at least one week washout period in between (Figure 1). During each visit, SCFA were administered either in the small intestine or the colon using standard capsules or capsules with a colon-delivery coating, respectively. Three days prior to each study visit, participants refrained from alcohol, followed a low-fibre diet (< 10g fibre/ day) and registered their dietary intake. On the evening prior to each study visit, subjects consumed a non-fermentable standard meal to avoid differences in colonic fermentation during the study visits.

On the morning of the study visit, participants arrived fasted at the study centre, and an intravenous catheter was inserted in the antecubital vein of both forearms. One catheter was used for blood collection, while the other was connected to a primed, continuous infusion that ran throughout the study visit (12 hours). The infusion contained deuterium (^2H)-labelled acetate, propionate and butyrate (20 µmol.kg^{-1}.h^{-1} [$^2H_3$]-acetate, 2 µmol µmol.kg^{-1}.h^{-1} [$^2H_5$]-propionate and 1 µmol.kg^{-1}.h^{-1} [$^2H_7$]-butyrate) and was applied to assess the clearance of SCFA in the blood circulation. The participants ingested 4 capsules (either uncoated or colon-delivery) containing ^{13}C-labelled SCFA (~400 mg ^{13}C-acetate, 300 mg ^{13}C-propionate and 800 mg ^{13}C-butyrate) during a standardised, fiber-free breakfast. Subsequently, blood samples were collected at regular time points for 12 hours. Participants consumed additional standardised, non-fermentable meals 4 and 10 hours after breakfast. At the end of the study visit, participants completed the gastrointestinal symptom rating scale (GSRS) to evaluate eventual gastrointestinal discomfort or symptoms during the study visit.

In each blood sample, total SCFA concentrations, as well as ^{13}C- and ^2H-abundance, were measured to calculate systemic availability. Total SCFA concentrations were determined following derivatisation with 2,4-difluoroaniline and extraction in ethyl acetate using gas chromatography-mass spectrometry [4]. For the analysis of ^{13}C- and ^2H-abundances, serum samples were deproteinised and extracted in diethyl ether using the method of Morrison et al. [5]. The ^{13}C-abundance was analysed in the extract as previously mentioned [6]. The same ether extract was then analysed for ^2H-abundance using gas chromatography coupled to a quadrupole mass spectrometer.

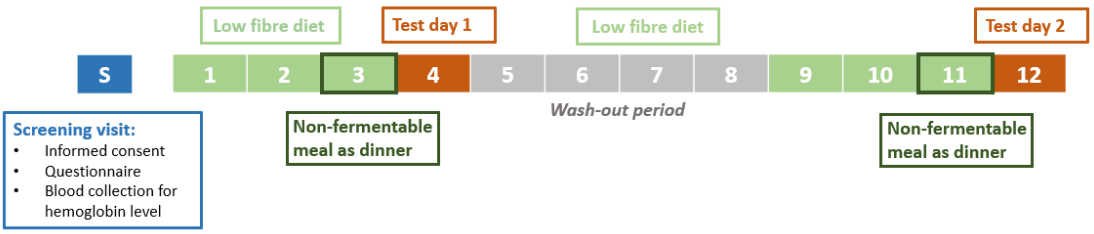


Figure 1: schematic overview of the study design of study 1

2.2 Study 2: the gut hormone release

Twenty-eight healthy volunteers (11 male /17 female; age 23 ± 4 years; BMI: 22 ± 2 kg/m²) conducted three study visits of each 8.5 hours with a minimum of one week washout period between each visit (Figure 2). During these visits, participants ingested either uncoated or colon-delivery capsules filled with unlabeled SCFA or placebo capsules filled with microcrystalline cellulose. Placebo capsules were used to account for the gut hormone release induced by the meals consumed during the visit. Three

days prior to each visit, participants refrained from alcohol, followed a low-fibre diet and logged their food intake. On the evening before each visit, participants consumed a standardised, non-fermentable meal to limit SCFA production due to colonic fermentation at the start of the visit.

On the morning of the study visit, participants arrived at the study centre after an overnight fast. An intravenous catheter was placed in the antecubital vein of the forearm for blood sampling. After baseline blood samples were collected, participants were offered a standard, no-fibre breakfast during which they ingested 30 of either the colon-delivery or uncoated capsules (~11.40 g sodium acetate, 5.10 g sodium propionate and 4.46 g of sodium butyrate, corresponding to about 20g of fermentable fibre) or placebo capsules. We deliberately administered a high dose of SCFA to maximise the chances of observing an effect on physiological parameters. Subsequently, blood samples were collected at regular time points for 8 hours for quantification of GLP-1, PYYSCFA, c-peptide and glucose concentrations. Upon every blood collection, participants rated their hunger, satiety, fullness and desire-to-eat on 100 mm Visual Analogue Scales (VAS) to evaluate the impact of the SCFA administration on subjective appetite. Four hours after breakfast, a non-fermentable, fibre-free lunch was served. At the end of the visit, participants completed the GSRS to record any gastrointestinal symptoms they might have experienced during the visit.

GLP-1 and PYY concentrations were measured with a commercially available Mesoscale Discovery (MSD) kit. Total SCFA concentrations were measured using gas chromatography-mass spectrometry [4]. Glucose and c-peptide concentrations were measured by the laboratory of the University Hospital of Leuven using standard laboratory techniques.

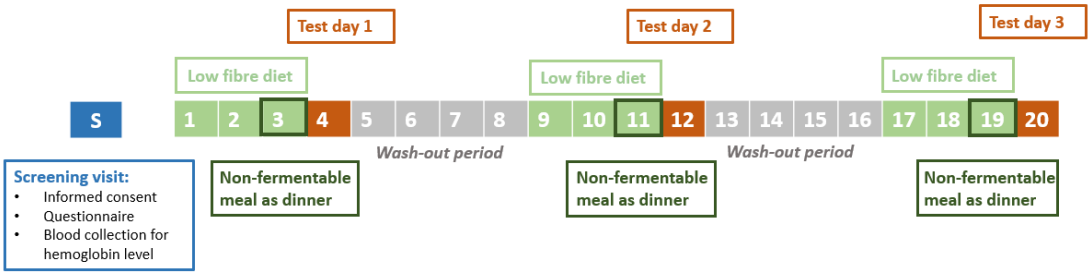


Figure 2: Schematic overview of study design of study 2

3. Results

List all results and describe them in all clarity and detail necessary; explain how your results help to achieve the general goals of either this WP or of the whole HealthFerm project.

3.1 Study 1: systemic availability

Systemic availability data were available for 9 subjects for acetate and 11 subjects for propionate and butyrate due to technical challenges encountered during sample analysis. Following small intestinal administration, peak concentrations of ^{13}C-labeled acetate, propionate and butyrate were reached within 30 minutes of capsule ingestion (Figure 3). With colonic administration, ^{13}C-SCFA concentrations began to increase between 3.5 and 4 hours and remained elevated for up to 7 hours after capsule ingestion.

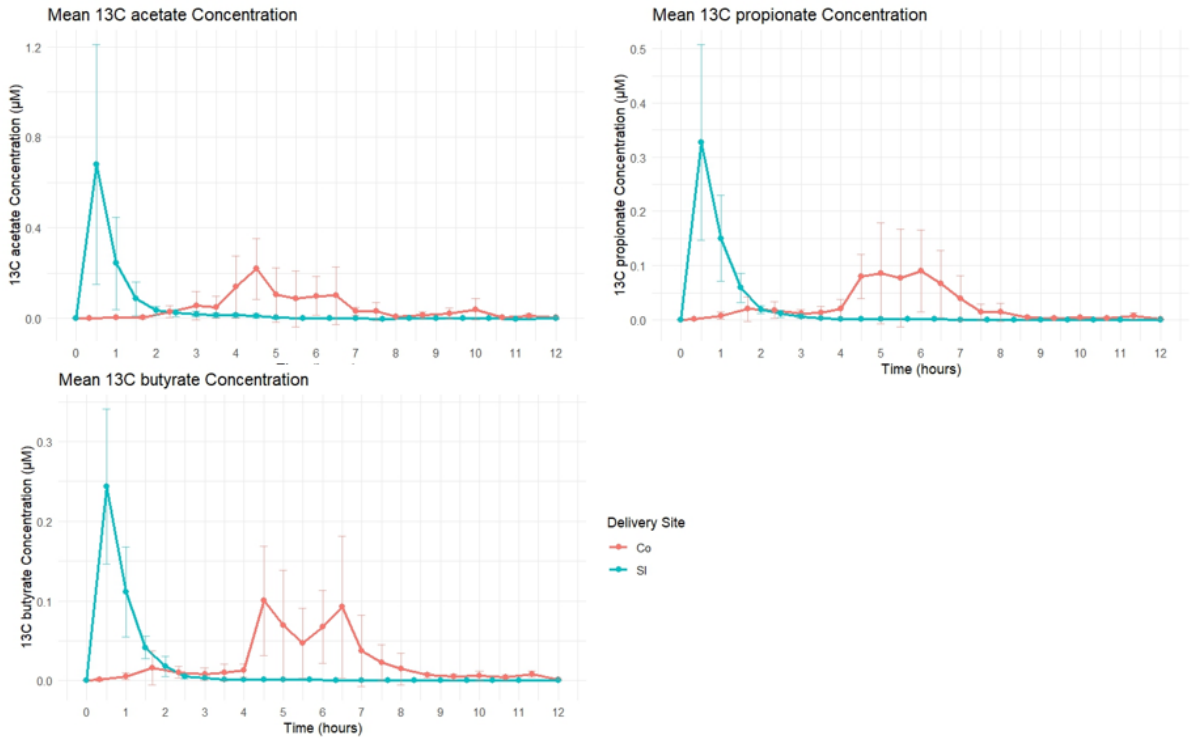
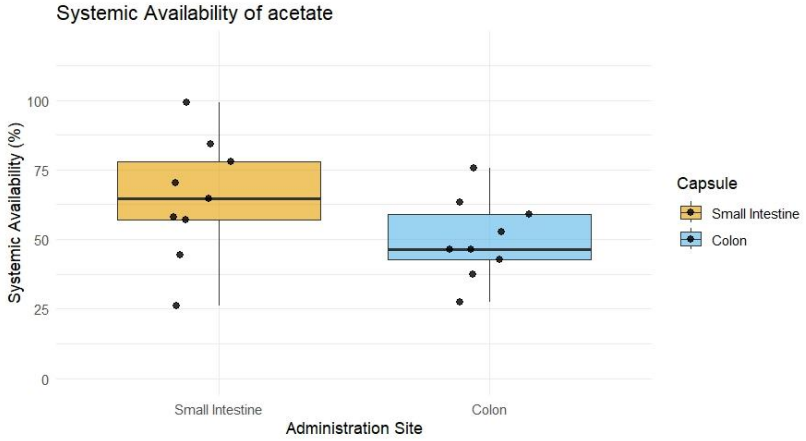


Figure 3: ^{13}C-acetate, ^{13}C-propionate and ^{13}C-butyrate concentrations over time following small intestinal or colonic administration.
Curves represent the mean ^{13}C-acetate (n = 9), ^{13}C-propionate and ^{13}C-butyrate concentrations (n = 11) over time. Error bars denote the 95% confidence interval. Co: colon delivery; SI: small intestinal delivery.

The systemic availability following small intestinal and colonic delivery was analysed using the Wilcoxon signed-rank test (Figure 4). The systemic availability of propionate was higher upon small intestinal administration compared to the colonic delivery (median [IQR]: 9.89% [7.68 – 11.46%] vs. 6.53% [4.99 – 8.46%], p = 0.024). In contrast, no significant differences were found for acetate (median [IQR]: 64.64% [57.03 – 77.93%] vs. 46.54% [42.58 – 59.1%], p = 0.16) and butyrate (median [IQR]: 3.19% [2.74 – 3.44%] vs. 2.26 [2.05 – 3.30%], p = 0.4131) between small intestinal and colonic delivery.



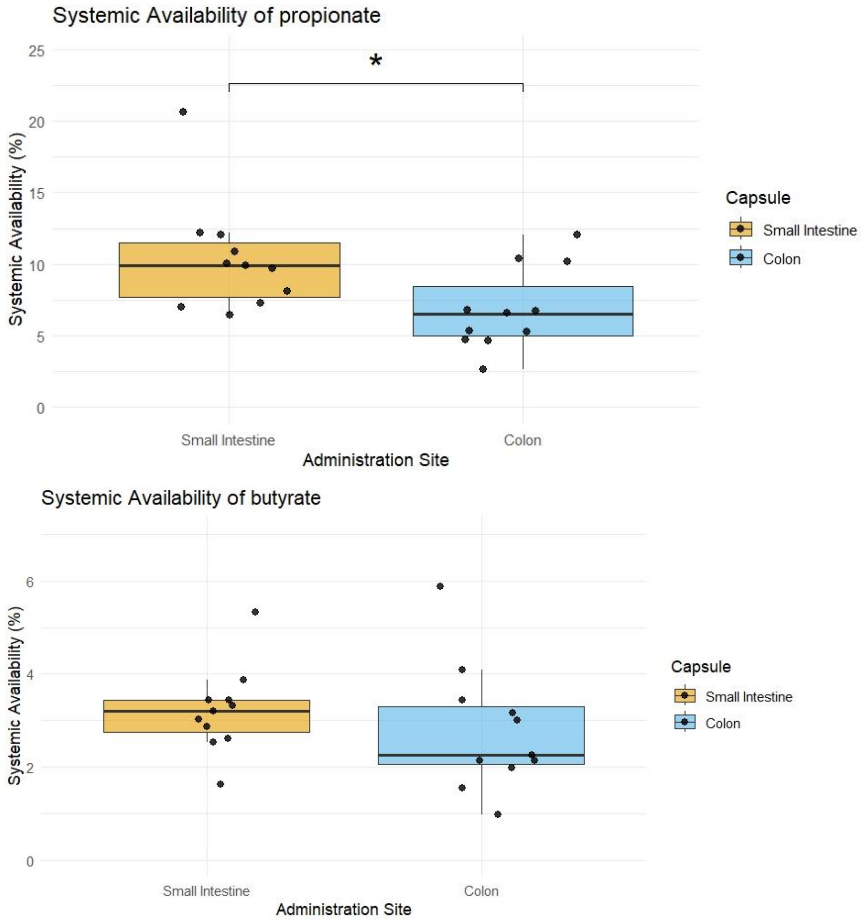


Figure 4: systemic availability (%) of acetate, propionate and butyrate following small intestinal and colonic administration.

Boxplots display the median and interquartile range (IQR) for the systemic availability of acetate (n = 9), propionate (n = 11) and butyrate (n = 11). Asterisk (*) indicates a statistically significant difference ($p < 0.05$).

3.2 Study 2: gut hormone release

The results of study 2 were statistically analysed using repeated measures linear mixed model with capsule, time and their interaction (capsule x time) as fixed effects and subject as a random effect. With GLP-1, we observed a capsule x time interaction effect ($F(1,1395)= 6.893$, $p = 0.00105$). The GLP-1 concentrations were higher at 1.5 hours ($p_{adj} = 0.0010$), 2 hours ($p_{adj} = 0.0007$) and 2.5 hours ($p_{adj} = 0.0076$) with small intestinal delivery compared to colonic delivery (Figure 5). Significant differences between small intestinal delivery and placebo, and colonic delivery and placebo were indicated in Figure 5. In addition, the capsule had a main effect on GLP-1 release ($F(2,1395)= 12.496$, $p < 0.0001$). This effect was further evaluated using post hoc planned contrasts. GLP-1 release was higher with small intestinal delivery compared to placebo ($\beta = 0.1505$, $SE = 0.0195$, $t(1395) = 7.725$, $p_{adj} < 0.0001$) and with colonic delivery compared to placebo ($\beta = 0.1057$, $SE = 0.0195$, $t(1395) = 5.242$, $p_{adj} < 0.0001$) but it was not different between small intestinal and colonic delivery ($\beta = 0.0448$, $SE = 0.0195$, $t(1395) = 2.302$, $p_{adj} = 0.0645$).

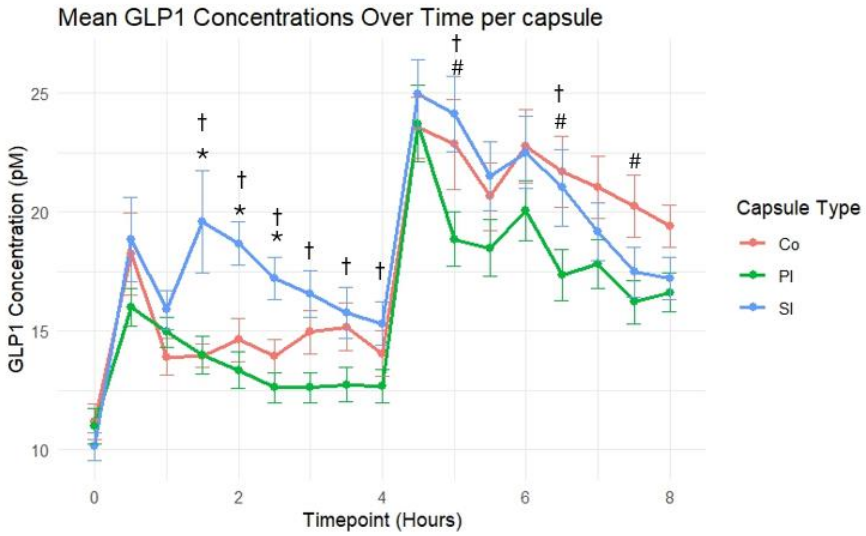


Figure 5: GLP-1 release over time per capsule type.
*Curves represent the mean GLP-1 concentration (pM) of 28 (n = 28) subjects. Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; SI: small intestinal delivery. * significant difference between colon and small intestine, # significant difference between colon and placebo, † significant difference between small intestine and placebo.*

We observed an interaction between capsule x time on PYY release ($F(2,1395) = 92.651$, $p < 0.0001$) (Figure 6). At 1.5 hours PYY concentrations were higher with small intestinal delivery compared to colonic delivery ($p_{adj} = 0.030$). In contrast, PYY release was higher between 6 and 8 hours upon colonic delivery compared to small intestinal delivery ($p_{adj} < 0.001$ at each time point). In addition, the PYY concentration was higher between 1.5 and 4 hours ($p_{adj} < 0.01$) and at 5 ($p_{adj} = 0.0003$) and 5.5 hours ($p_{adj} = 0.0246$) with small intestinal delivery and between 4 and 8 hours with colonic delivery compared to placebo ($p_{adj} = 0.0354$ at 4 hours, $p_{adj} = 0.0060$ at 4.5 hours and $p_{adj} < 0.0001$ for each timepoint between 5 and 8 hours). For PYY, we also detected a capsule effect ($F(2,1395) = 30.057$, $p < 0.0001$), which was further evaluated using post hoc planned contrasts. PYY release was higher with colonic delivery compared to small intestinal delivery ($\beta = 0.109$, $SE = 0.0173$, $t(1395) = 6.332$, $p_{adj} < 0.001$), with colonic delivery compared to placebo ($\beta = 0.260$, $SE = 0.0173$, $t(1395) = 15.053$, $p_{adj} < 0.001$) and with small intestinal delivery compared to placebo ($\beta = 0.151$, $SE = 0.0173$, $t(1395) = 8.721$, $p_{adj} < 0.001$).

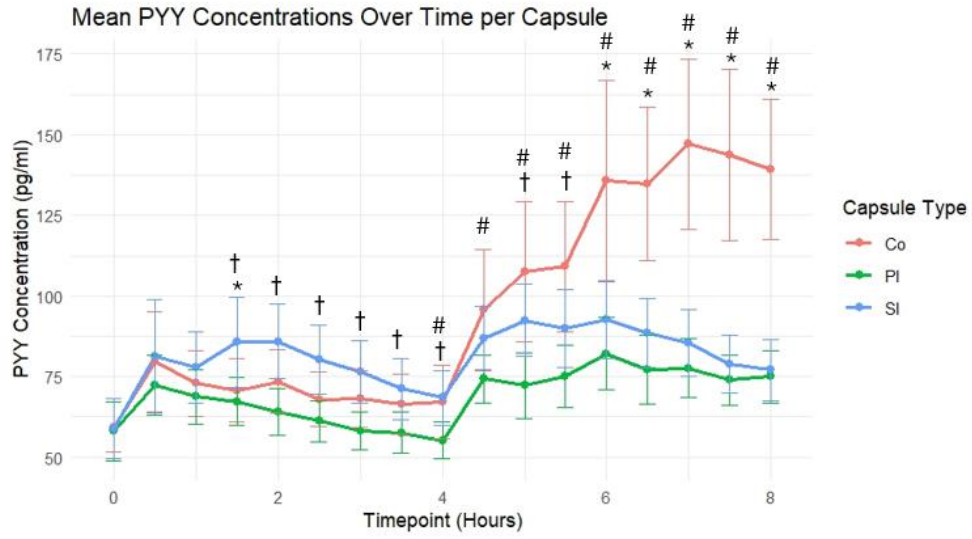


Figure 6: PYY release over time per capsule type.
*Curves represent the mean PYY concentration (pg/ml) of 28 subjects (n = 28) over time. Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; SI: small intestinal delivery. * significant difference between colon and small intestine, # significant difference between colon and placebo, † significant difference between small intestine and placebo, p < 0.05.*

There was no interaction between capsule x time on glucose (F(2,723) = 0.0132, p = 0.9869) and c-peptide release (F(2,723) = 0.0503, p = 0.9509) (Figure 7 and 8). Furthermore, there was no difference in glucose and c-peptide release between small intestinal delivery, colonic delivery and placebo capsules (p = 1 for each pairwise comparison).

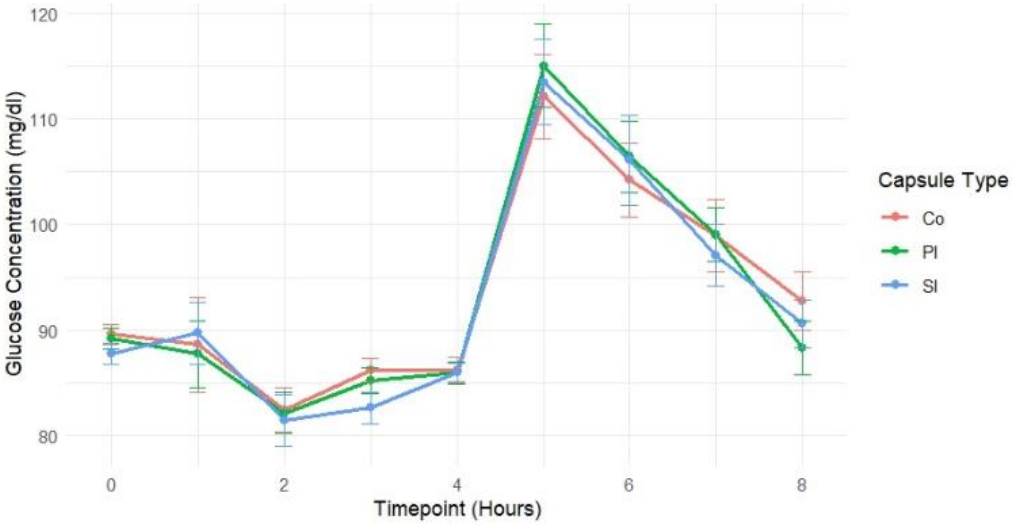


Figure 7: Glucose release over time per capsule.
Curves represent mean glucose concentration (mg/dl) of 28 subjects over time. Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; SI: small intestinal delivery.

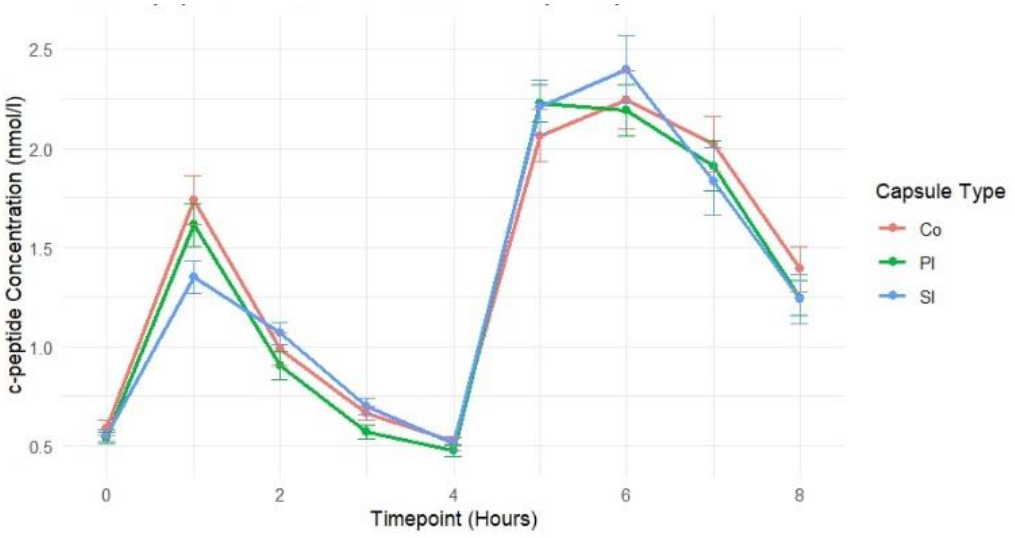
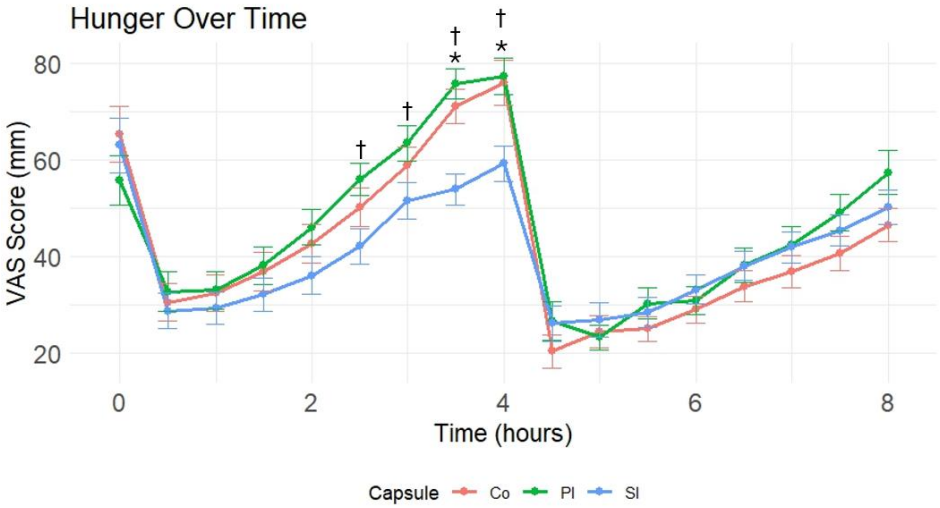
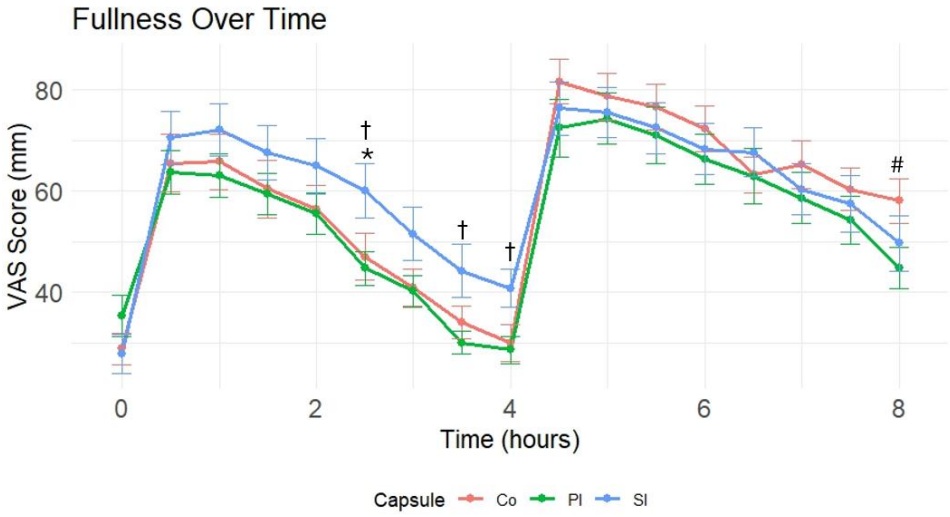


Figure 8: c-peptide release over time per capsule.
Curves represent mean c-peptide concentration (nmol/l) of 28 subjects (n= 28) over time. Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; SI: small intestinal delivery.

We found an interaction between capsule x time and hunger (F(2,1395) = 3.2579, p = 0.0388), fullness (F(2, 1395) = 4.0432, p_{adj} = 0.0177) and desire-to-eat (F(2,1395) = 5.46, p_{adj} = 0.00436), but not with

satiety (F (2,1395) = 2.31, p = 0.099). Significant differences at specific time points between small intestinal delivery, colonic delivery and placebo for hunger, fullness and desire-to-eat are indicated in Figure 9. Using follow up planned contrasts we could conclude that administration of SCFA in the small intestine led to higher fullness (β = 6.02, SE = 1.49, t(1395) = 4.027, p_{adj} = 0.0002), higher satiety (β = 5.09, SE = 1.46, t(1395) = 3.477, p_{adj} = 0.0016) and less desire-to-eat (β = -7.28, SE = 1.48, t(1395) = 4.909, p_{adj} < 0.0001) compared to colonic delivery. In addition, participants reported higher satiety (β = 3.88, SE = 1.46, t(1395) = 2.648, p_{adj} = 0.0246) and less desire-to-eat (β = -5.79, SE = 1.48, t(1395) = -3.905, p_{adj} = 0.0003) with colonic delivery compared to placebo. Compared to placebo, small intestinal administration led to lower hunger scores ((β = -5.32, SE = 1.51, t(1395) = -3.524, p_{adj} = 0.0013).





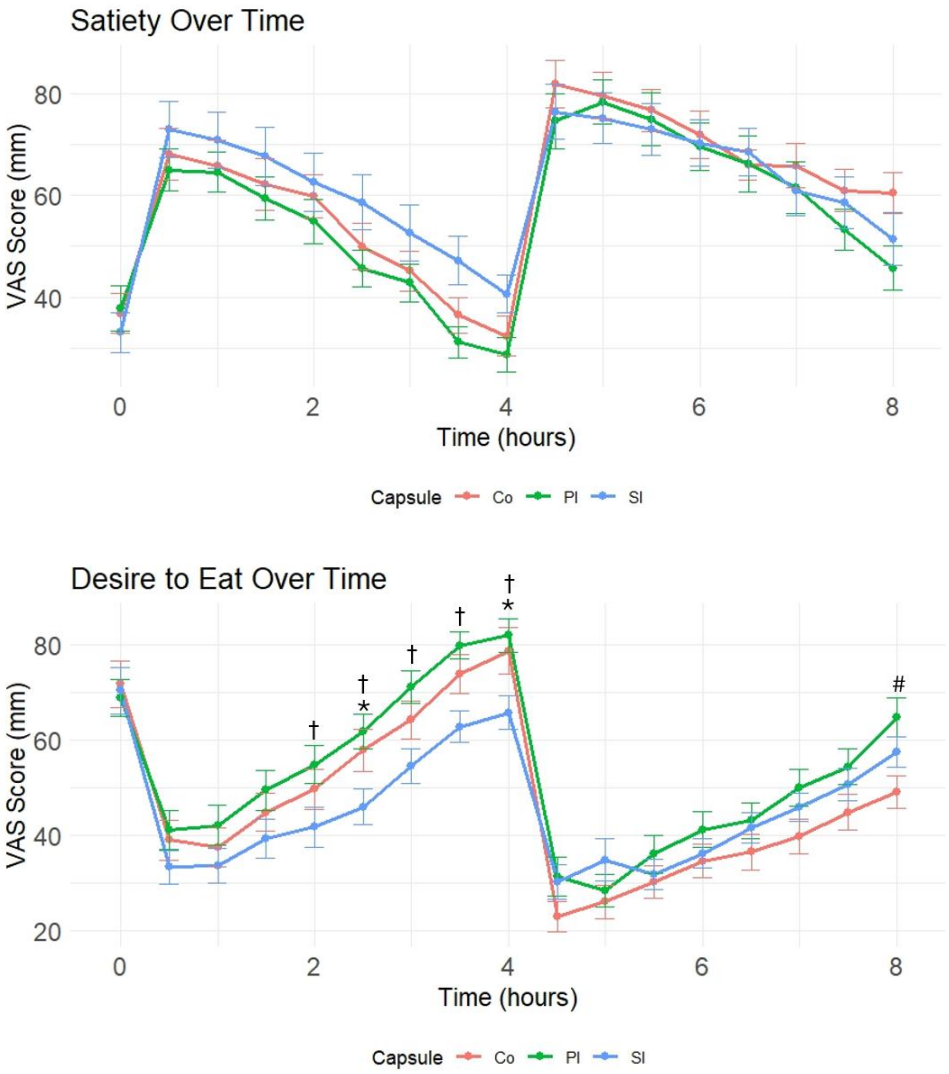


Figure 9: VAS scores for hunger, fullness, satiety and desire-to-eat over time per capsule.
*Curves represent the mean VAS score for hunger, fullness, satiety and desire-to-eat of 28 subjects (n = 28) over time. Error bars indicate the 95% confidence interval. Co: colon delivery; PI: placebo; SI: small intestinal delivery. * significant difference between colon and small intestine, # significant difference between colon and placebo, † significant difference between small intestine and placebo, p < 0.05.*

A capsule*time interaction effect was observed for acetate ($F(2,723) = 77.33$, $p < 0.0001$), propionate ($F(2,723) = 54.26$, $p < 0.0001$) and butyrate concentrations ($F(2,723) = 34.72$, $p < 0.0001$) (Figure 10,11 and 12). With small intestinal delivery, acetate, propionate and butyrate concentrations increased immediately upon capsule ingestion and were higher between 1 and 3 hours compared to colonic delivery. With colonic delivery, SCFA concentrations increased within 2 to 4 hours and were higher between 5 and 8 hours compared to small intestinal delivery. SCFA concentrations were higher between 1 and 4 hours with small intestinal delivery compared to placebo and between 3 and 8 hours with colonic delivery compared to placebo.

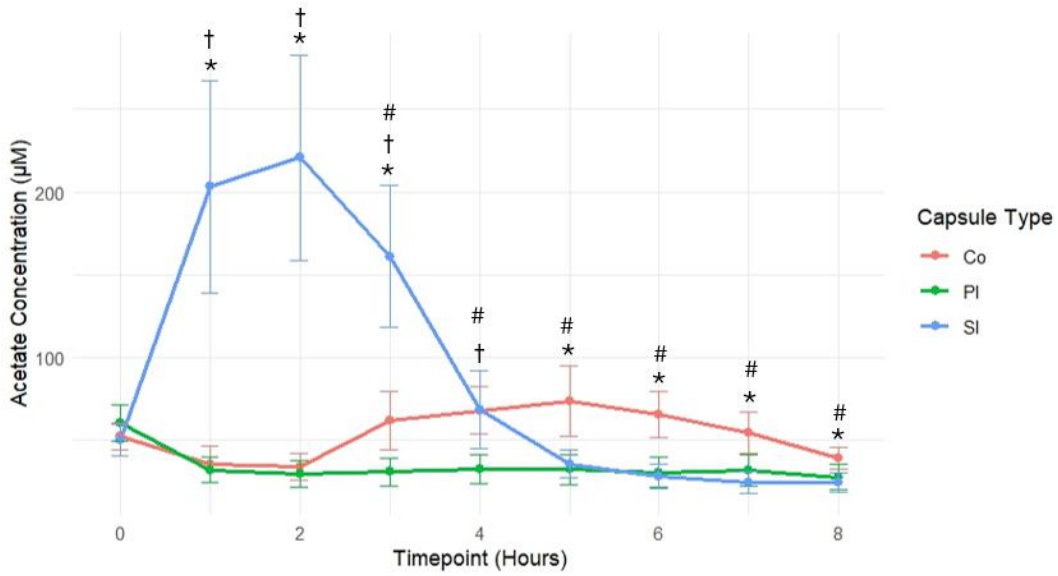


Figure 10: acetate concentration (µM) over time per capsule.
*Curves represent the mean acetate concentration (µM) of 28 subjects (n = 28). Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; SI: small intestinal delivery. * significant difference between colon and small intestine, # significant difference between colon and placebo, † significant difference between small intestine and placebo, p < 0.05.*

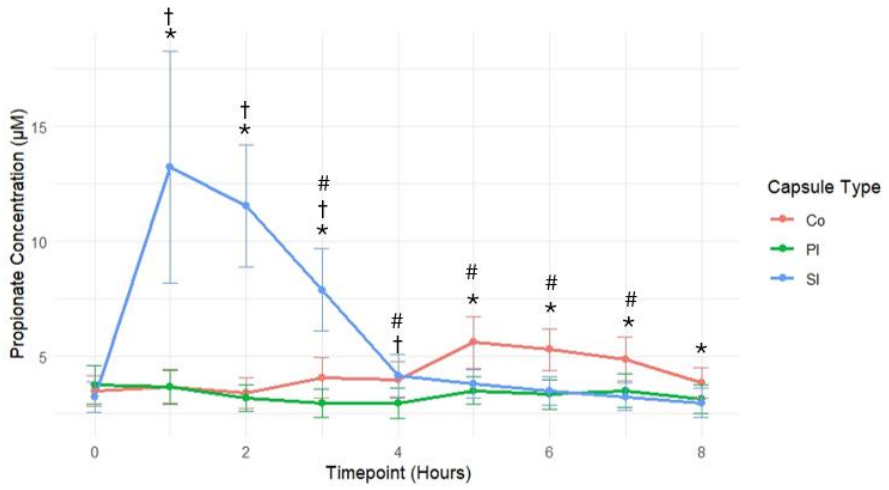


Figure 11: propionate concentration (µM) over time per capsule.
*Curves represent the mean propionate concentration (µM) of 28 subjects (n=28). Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; SI: small intestinal delivery. * significant difference between colon and small intestine, # significant difference between colon and placebo, † significant difference between small intestine and placebo, p < 0.05.*

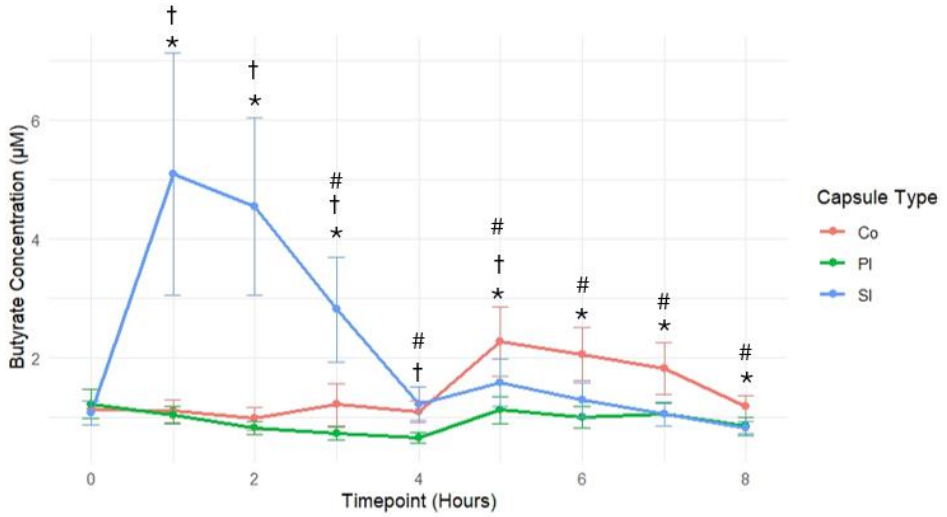


Figure 12: butyrate concentration (µM) over time per capsule.
*Curves represent the mean butyrate concentration (µM) of 28 subjects (n=28). Error bars indicate the 95% confidence interval. Co: colon delivery; Pl: placebo; Sl: small intestinal delivery. * significant difference between colon and small intestine, # significant difference between colon and placebo, † significant difference between small intestine and placebo, p < 0.05.*

4. Conclusion

Please explain the meaning and the relevance of your results. If applicable also comment on any deviation(s). Boldface important parts

In the first study, ^{13}C-SCFA concentrations peaked within 30 minutes following small intestinal delivery, while with colonic delivery, ^{13}C-SCFA entered the blood circulation between 3.5 and 4 hours upon capsule ingestion. This observation confirmed the targeted release of SCFA in either the small intestine or the colon using the targeted delivery capsules. The systemic availability of propionate was higher following small intestinal compared to colonic administration. However, no significant differences were observed for acetate and butyrate. Thus, our hypothesis was confirmed only for propionate. It is important to mention that after small intestinal delivery of the SCFA, systemic concentrations increased after 30 min and returned rapidly back to baseline after 60 min. It is highly likely that the concentration at 30 min was not the peak concentration and that, therefore, our calculation of systemic availability is underestimated. More frequent blood sampling in the first hour after SCFA administration to the small intestine might have been preferred. Yet, the need for 10 ml blood at each time point to allow for all required measurements hampered such frequent sampling.

In the second study, both administration of SCFA in the small intestine and in the colon increased GLP-1 release, although the amount of GLP-1 was not different depending on the site of administration. PYY release was also higher upon SCFA administration relative to placebo and was higher upon colonic compared to small intestinal delivery. Secondly, we investigated whether the SCFA-induced hormone release also resulted in clinically relevant effects. Whereas the release of GLP-1 and PYY did not affect glucose homeostasis (glucose and c-peptide concentrations did not differ between colonic, small intestinal delivery of SCFA and placebo), targeted delivery of SCFA did have an impact on appetite regulation. Specifically, the hunger score was lower with small intestinal SCFA administration compared to placebo and colonic SCFA delivery led to higher satiety and reduced desire-to-eat compared to placebo. Additionally, administration of SCFA in the small intestine also increased fullness and satiety while reducing the desire-to-eat compared to colonic SCFA delivery.

Since the dose of SCFA administered in study 2 was higher than in study 1, SCFA concentrations remained elevated in blood up to 3 hours after small intestinal administration and between 4 and 8 hours following colonic administration, supporting the targeted release of SCFA with the standard and colon delivery capsules. Systemic concentrations of acetate, propionate and butyrate were higher following small intestinal administration compared to colonic delivery. Although these results do not allow a formal calculation of the systemic availability (no data on clearance), this observation suggests that the hypothesis of increased systemic availability following small intestinal administration compared to colonic administration may still be valid, also for acetate and butyrate.

5. Deviations, if applicable

Please refer to any deviations from the originally planned work related to this deliverable (e.g. content, timing etc.). Please explain briefly any impact this might have on other parts of the action like other work packages, deliverables or milestones.

KU Leuven made the decision to evaluate the two outcomes in two separate studies to allow for the selection of the most optimal study design for each objective. As a result, this deliverable was completed 6 months later than initially anticipated (with the agreement of the project officer). However, this delay does not have any impact on other work packages, deliverables or milestones.

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