Colonic Delivery of $\alpha$-Linolenic Acid by an Advanced Nutrient Delivery System Prolongs Glucagon-Like Peptide-1 Secretion and Inhibits Food Intake in Mice

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Scope: Nutrients stimulate the secretion of glucagon-like peptide-1 (GLP-1), an incretin hormone, secreted from enteroendocrine L-cells which decreases food intake. Thus, GLP-1 analogs are approved for the treatment of obesity, yet cost and side effects limit their use. L-cells are mainly localized in the distal ileum and colon, which hinders the utilization of nutrients targeting GLP-1 secretion. This study proposes a controlled delivery system for nutrients, inducing a prolonged endogenous GLP-1 release which results in a decrease food intake.

Methods and Results: $\alpha$-Linolenic acid ($\alpha$LA) was loaded into thermally hydrocarbonized porous silicon (THCPSi) particles. In vitro characterization and in vivo effects of $\alpha$LA loaded particles on GLP-1 secretion and food intake were studied in mice. A total of 40.4 ± 3.2% of loaded $\alpha$LA is released from particles into biorelevant buffer over 24 h, and $\alpha$LA loaded THCPSi significantly increased in vitro GLP-1 secretion. Single-dose orally given $\alpha$LA loaded mesoporous particles increased plasma active GLP-1 levels at 3 and 4 h and significantly reduced the area under the curve of 24 h food intake in mice.

Conclusions: $\alpha$LA loaded THCPSi particles could be used to endogenously stimulate sustain gastrointestinal hormone release and reduce food intake.

1. Introduction

Obesity and overweight are major risk factors for various diseases including type 2 diabetes mellitus (T2DM), and their prevalence almost tripled since 1975.[1] Hence, novel prevention and treatment methods for obesity are urgently needed.

After a meal, digested food components such as amino acids, saccharides, and fatty acids (FAs) stimulate the secretion of several gut peptides in the gastrointestinal (GI) tract.[2] Glucagon-like peptide-1 (GLP-1) is an incretin hormone, secreted from the enteroendocrine L-cells of the distal ileum and colon in response to nutrient ingestion.[2] It plays a significant role in insulin secretion from pancreatic $\beta$-cells, delays gastric emptying, and induces satiety via the gut–brain axis.[2,3] Due to the important physiological functions, GLP-1 has been targeted for the treatment of T2DM and obesity.[4,5] The USFDA approved GLP-1 agonists for the treatment of obesity,
indicating the importance of GLP-1 pathway in the treatment of obesity.\textsuperscript{[6]} Even though GLP-1 agonists are in clinical use, those are only applied to T2DM or obese subjects. The high cost of the medication and side effects prevent their availability for broad use. While GLP-1 works as an effective appetite regulator, it has a very short half-life (<2 min) due to inactivation by dipeptidyl peptidase-4 (DPP4). Less than 15% of secreted GLP-1 reaches the systemic circulation.\textsuperscript{[7]} In addition, GLP-1 secreting L-cells are mainly located in the distal part of the GI tract, which most of the nutrients do not reach.\textsuperscript{[8]} These difficulties hinder the utilization of nutritional compounds for additional endogenous stimulation of GLP-1 release. Thus, a novel delivery system for nutritional compounds to the distal part of the GI tract may sustain endogenous GLP-1 release and thus prolong food intake inhibition.

\(\alpha\)-Linolenic acid (\(\alpha\)LA) was selected as a model nutrient to stimulate endogenous GLP-1 release. \(\alpha\)LA is an essential omega-3 long-chain fatty acid (LCFA), the precursor of eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which have critical roles in brain development, cardiovascular health, and inflammatory responses.\textsuperscript{[9]} The most common daily sources of \(\alpha\)LA are vegetable and seed oils such as flaxseed, canola, and soybean oils. Various in vitro and in vivo studies have demonstrated that \(\alpha\)LA stimulates GLP-1 secretion via a G protein-coupled receptor 120 (GPR120).\textsuperscript{[10,11]} In previous studies, we have shown that \(\alpha\)LA and \(\alpha\)LA loaded THCSi particles increased GLP-1 secretion from enteroendocrine cell lines, STC-1, and GLUTag.\textsuperscript{[12,13]}

Porous silicon (PSi) particles are safe and nontoxic and have been studied as a carrier material for drug delivery.\textsuperscript{[14,15]} Due to a simple and gentle loading procedure, PSi particles can be loaded with relatively high amounts of unstable compounds.\textsuperscript{[16]} PSi particles can be subjected to chemical modification to optimize the interaction with the payload.\textsuperscript{[17,18]} In this study, thermally hydrocarbonized PSi (THCPSi) particles were selected as the carrier material because THCPSi surface is covered by hydrocarbons, which provides low wettability and hydrophobic properties.\textsuperscript{[19]} This feature allows the loading of hydrophobic molecules such as FAs and to release the payload gradually.\textsuperscript{[20,21]} Several previous studies have demonstrated excellent stability, non-cytotoxicity, and non-immune reactivity of THCPSi particles in vitro and in vivo, indicating their suitability as an oral delivery material.\textsuperscript{[12,22,23]}

We hypothesized that THCPSi particles deliver \(\alpha\)LA to the distal part of the GI tract and inhibit food intake via stimulating GLP-1 secretion by the released \(\alpha\)LA. The particles need to be stable and able to release loaded nutrients continuously, thus we first evaluated the characteristic of particles and release profiles of \(\alpha\)LA loaded particles in biorelevant buffers considering oral administration. Then, the effects of \(\alpha\)LA loaded particles on GLP-1 secretion from enteroendocrine cells, and the plasma GLP-1 levels and food intake of mice after single-dose oral administration of \(\alpha\)LA loaded particles were studied.

\section*{2. Results}

\subsection*{2.1. Characterization of THCPSi Particles and Thermogravimetric Analysis (TG)}

The silicon hydride terminated surfaces of the PSi microparticles were passivated with thermal hydrocarbonization and the surface replaced with short hydrocarbons (Figure S1A, Supporting Information), as previously demonstrated.\textsuperscript{[24]} The THCPSi microparticles (Figure 1) have a mesoporous nature (Figure S1B,C, Supporting Information).\textsuperscript{[25]} The specific surface area was 295 ± 4 m\(^2\) g\(^{-1}\), with a pore volume of 0.94 ± 0.01 cm\(^3\) g\(^{-1}\) corresponding to a porosity of ca. 69% and an average pore diameter of 13 ± 1 nm. The \(\alpha\)LA loaded THCPSi particles were prepared in batches named as THCPSi 1–5. The actual loading degrees of those batches were 19.0 ± 0.3% (mean ± SEM, \(n = 5\)), examined by TG (Figure S2, Supporting Information).

\subsection*{2.2. Release Characteristics of \(\alpha\)LA from THCPSi Particles}

In vitro release of \(\alpha\)LA from THCPSi particles into biorelevant media is shown in Figure 2A. The released amount of \(\alpha\)LA into fasted state simulated gastric fluid (FaSSGF) in the first 1 h was 15.3 ± 2.5% of the total loaded amount. The media was then changed to FaSSIF for the following 1 h with \(\alpha\)LA release of 6.7 ± 0.5%. When the media was altered into blank fasted state simulated intestinal fluid (FaSSIF) for the next 2 h, 13.5 ± 1.0% of the loaded \(\alpha\)LA was released. Lastly, the released amount of \(\alpha\)LA into simulated colonic fluid (SCoF) for the following 20 h was 4.9 ± 0.8%. Totally 40.4 ± 3.2% of \(\alpha\)LA was released in 24 h, and release mechanism followed Fickian diffusion (\(n = 0.356 ± 0.03\), \(n = 4\)) with an average release rate of 9.9 ± 2.2% h\(^{-1}\). The release rate was highest during the first 30 min (26.1 ± 3.5% h\(^{-1}\)), and gradually decreased along with the incubation time (Figure 2B). The unreleased \(\alpha\)LA (19.3 ± 2.5%) was measured from hexane-extracted solution (data not shown). The detected amount of \(\alpha\)LA by HPLC from positive controls are shown in Figure 2C, indicating that the measurable concentration of \(\alpha\)LA was significantly decreased after 6 h, and only 23.7 ± 5.4% of \(\alpha\)LA was detected after 24 h incubation compared to samples after 1 h of incubation.

\subsection*{2.3. In vitro GLP-1 Secretion with \(\alpha\)LA Loaded THCPSi Particles in STC-1 Cells}

50 \(\mu\)M of \(\alpha\)LA was used as such or loaded in THCPSi particles (19% loading) to study in vitro GLP-1 release from STC-1

Figure 1. Characterization of THCPSi particles. Secondary electron micrograph of the THCPSi particles.
cells. Both αLA and αLA loaded THCSi particles significantly increased GLP-1 secretion from STC-1 cells being 2.8 and 3.7 times higher than that of the control, respectively (Figure 2D). The empty THCSi particles had no effect on GLP-1 secretion. From 2 h incubated samples of 50 μM αLA and 19% αLA loaded THCSi particles, 43.1 ± 1.8 and 29.8 ± 1.3 μM of αLA were detected by HPLC, respectively.

### 2.4. Dose Response Effect of αLA on the Food Intake in Mice

To study the effect of αLA on the food intake in mice, three doses of αLA (100, 200, and 500 mg kg⁻¹) were orally administered to mice. 24 h food intake in mice, and its AUC was calculated (Figure 3A,B). Both 200 and 500 mg kg⁻¹ doses of αLA significantly reduced AUC of 24 h food intake in mice with 16.5% and 17.3% respectively compared to control, while 100 mg kg⁻¹ was ineffective. No significant difference was observed in water intake (24 h) (Figure S3A, Supporting Information), 25–48 h food intake (Figure S3B, Supporting Information), total activity, energy expenditure, O₂ consumption (VO₂), CO₂ production (VCO₂), and average respiratory exchange ratio (RER), in both the dark (1–13 h) and light (14–26 h) phases in mice (Figure 3C–G). The activity and energy expenditure of mice from −24 to 48 h are shown in Figure S3C,D (Supporting Information), respectively. In addition, no significant change was detected in AUC of 24 h food intake between the two higher doses of αLA 200 and 500 mg kg⁻¹. Based on these results, αLA 200 mg kg⁻¹ (lowest effective dosage) was selected as a reference dosage for further experiments.

### 2.5. Effect of αLA Loaded THCSi Particles on the Plasma Active GLP-1 Level in Mice

The plasma active GLP-1 levels after oral dosing of samples are shown in Figure 4A. αLA loaded THCSi significantly increased...
Figure 3. αLA inhibited the food intake in mice. A) 24 h food intake of mice after oral administration of control or αLA (100, 200, and 500 mg kg\(^{-1}\)), and B) AUC of 24 h food intake. C) Total activity, D) Energy expenditure, E) \(\text{VO}_2\), F) \(\text{VCO}_2\), and G) Average RER after oral gavage during the dark (1–13 h) and the light (14–26 h) phases. Box represents the median, 25th–75th percentiles, and whiskers show the minimum and maximum values. Statistical significance was assessed by two-way ANOVA with Dunnett’s multiple comparisons test A, C–G), and one-way ANOVA with Dunnett’s multiple comparisons test B): ** \(P < 0.01\). The values represent the mean ± SEM for \(n = 6\).
the plasma active GLP-1 level by 1.5 times at 3 h and 2.7 times at 4 h compared to control, whereas empty THCPSi particles did not. While glucose significantly elevated the plasma active GLP-1 level at 5 and 15 min with 7.6 and 2.4 times higher than control, respectively. αLA also increased plasma GLP-1 level after dosing with significant difference at 15 min. The AUC of plasma GLP-1 concentration is demonstrating that αLA loaded THCPSi significantly augmented total plasma active GLP-1 levels compared to control (Figure 4B). No significant difference was observed in blood glucose level up to 60 min after gavage except glucose at 5 min (Figure S4, Supporting Information).

2.6. Effect of αLA Loaded THCPSi Particles on the Food Intake in Mice

To examine the effect of THCPSi particles on the food intake in mice, αLA loaded THCPSi particles were prepared and administered via oral gavage to mice. 24 h food intake and AUC are shown in Figure 5A,B. The AUC of 24 h food intake of αLA and αLA loaded THCPSi were significantly reduced 28.5%, and 16.7% respectively, compared to control. There was no significant difference between αLA and αLA loaded THCPSi in 24 h total food intake, indicating that they have equal efficacy in the food intake inhibition. αLA inhibited food intake immediately, whereas αLA loaded THCPSi particles showed its effect approximately 5 h after oral dosing. No significant change in 24 h food intake was observed with empty THCPSi particles. Furthermore, there were no significant differences in 24 h water intake (Figure S5A, Supporting Information), 25–48 h food intake (Figure S5B, Supporting Information), and total activity in both the dark (1–13 h) and the light (14–26 h) phases among the groups (Figure S5C), whereas the energy expenditure, VO₂, VCO₂, and average RER of αLA 200 mg kg⁻¹ in the dark phase was significantly lower compared to control (Figure 5D–G). Figure 5H,I showed the activity and energy expenditure of mice from −24 to 48 h.

3. Discussion

We demonstrated that oral administration of αLA loaded THCPSi stimulated GLP-1 release in a sustained manner and inhibited the food intake in mice. LCFA, including αLA, stimulate GLP-1 secretion by activating GPR120.[10,11] However, oral delivery of FAs is difficult because they are absorbed before reaching the target site and therefore would require a delivery system to reach the distal part of the GI tract. We used PSi particles as a carrier for αLA delivery, because of their advantages as a carrier material.[16] The loaded αLA was released from carrier particles by diffusion in line with our previous results (Figure 2A).[12] The highest αLA release (13.1 ± 1.8%) was recorded during the first 30 min of incubation, which was probably due to αLA release from the external surface of the particles (Figure 2B).[26] The detectable levels of dissolved αLA decreased during longer incubation times at 6, 10, and 24 h (Figure 2C). These results indicated that mesoporous particles are not only carriers but also protector of loaded nutrients from the external environment preventing rapid oxidation.[27] Furthermore, FAs have several shortcomings for direct use as therapeutic materials, for instance, bitter taste and unpleasant smell. Thus, PSi particles can mask these deficiencies and improve the utility of FAs for an oral delivery system.

In mice, the transit time from the stomach to the small intestine, cecum, and colon has been reported to be 1, 3, and 6 h, respectively.[28] In addition, our in vitro results showed that both αLA and αLA loaded THCPSi particles stimulated GLP-1 secretion (Figure 2D). Based on these observations, αLA loaded...
Figure 5. αLA loaded THCPsi inhibited the food intake in mice. A) 24 h food intake of mice after oral administration of control, αLA 200 mg kg⁻¹, empty THCPsi, or 19% αLA loaded THCPsi, and B) AUC of 24 h food intake. C) Total activity. D) energy expenditure, E) VO₂, F) VCO₂, and G) average RER after oral gavage during the dark (1–13 h) and light (14–26 h) phases. Box represents the median, 25th–75th percentiles, and whiskers show the minimum and maximum values. H) Activity and I) Energy expenditure of mice from 24 h prior to 48 h after sample administration. Statistical significance was assessed by two-way ANOVA with Dunnett’s multiple comparisons test A, C–G), and one-way ANOVA with Dunnett’s multiple comparisons test B): *p < 0.05, **p < 0.01, ****p < 0.0001. The values represent the mean ± SEM for n = 6 (control, αLA, and αLA loaded THCPsi) and n = 7 (empty THCPsi).
αLA loaded THCPSi particles would be able to release the loaded αLA in the distal part of the GI tract where enteroendocrine L-cells are highly localized and induce GLP-1 secretion in a sustained manner. Other peptides released from the upper GI tract could contribute to the anorexigenic effects like cholecystokinin,[2] yet we targeted the distal part with our particles. We then tested our hypothesis in our follow-up in vivo experiments.

αLA loaded THCPSi particles significantly increased active GLP-1 levels in plasma 3 and 4 h after dosing compared to control, while αLA in solution stimulated plasma GLP-1 right after sample administration (Figure 4A). Furthermore, αLA loaded THCPSi had stronger stimulation of total GLP-1 than single dosing of glucose or αLA (Figure 4B), indicating that αLA loaded mesoporous particles were more effective to prolong the GLP-1 secretion than plain αLA or glucose in our experimental conditions. αLA has a high affinity for GPR120, which is widely expressed in the GI tract from the ileum to the rectum.[29,30] Thus, released αLA from particles binds to GPR120 and stimulates the release of appetite-regulating peptides, resulting in suppressing the food intake in mice. Indeed, administering 200 mg kg⁻¹ αLA as such and in 19% αLA loaded THCPSi particles reduced the AUC of 24 h food intake in mice (Figure 5B). Importantly, αLA

**Figure 5. Continued**
loaded mesoporous particles showed an anorexigenic effect approximately 5 h after oral dosing, while αLA inhibited food intake immediately (Figure 5A). This suggests that THCPsi particles released loaded αLA gradually while traveling through the GI tract of mice, and the anorexigenic effect was observed when particles reached the distal part of the intestine. Recently, Ichimura et al.\(^{[31]}\) demonstrated that a high-fat diet fed GPR120-deficient mice had advanced obesity and glucose intolerance. Moreover, in humans, a genetic polymorphism of GPR120 (R270H) lacks the ability to mediate the LCFA signals, which increases the risk of obesity and insulin resistance in European residents,\(^{[31]}\) indicating that GPR120 is a key player of FAs-mediated physiological responses in both rodents and humans.

Energy expenditure, \(O_2\) consumption, \(CO_2\) production, and average RER in the dark phase (1–13 h) were reduced after αLA administration (Figure 5D–G). The energy expenditure of αLA 200 mg kg\(^{-1}\) was suppressed up to 4 h after sample administration (Figure 5I). Even though no significant differences were observed in the metabolic parameters in dose–response study with αLA (Figure 3D–G), combined data of two identical experiments demonstrated significant differences between αLA and control in energy expenditure, \(CO_2\) production, and average RER (Figure S6A–E, Supporting Information). Increased locomotor activity and \(O_2\) consumption were reported in GLP-1 receptor knockout (DIRKO) mice on either chow or high-fat diet.\(^{[32]}\) These results imply that the effects of GLP-1 receptor knockout mice; \(\alpha\)LA and glucose-dependent insulinotropic polypeptide (GIP) receptor knockout mice; \(\alpha\)Gipr\(^{-/-}\) and its double incretin receptor knockout (DIRKO) mice on either chow or high-fat diet.\(^{[32]}\) These results imply that the effects of GLP-1 and GLP-1 receptor on regulating energy intake might be also associated with motor activity.

Our results demonstrate that oral administration of \(\alpha\)LA loaded THCPsi particles inhibit food intake via stimulating and prolonging GLP-1 secretion in mice, suggesting that mesoporous particles could serve as a novel carrier for applying sustained nutrient release in appetite regulation.

4. Experimental Section

Materials: Hydrofluoric acid (HF), \(\alpha\)-Linolenic acid, DPP4 inhibitor, DMEM, pancreatic, pepsin, sodium taurocholate hydrate, DMSO, carboxymethylcellulose sodium (CMC), sitagliptin phosphate monohydrate, and GLP-1 (active) ELISA kit (EGLP-35K) for in vitro GLP-1 analysis were purchased from Merck (Germany). Methanol, cell culture inserts, fetal bovine serum (FBS), horse serum, penicillin-streptomycin, glutamine were acquired from Thermo Fisher Scientific Inc. (Denmark). D(-)-Glucose was obtained from VWR International Oy (Finland), and EDTA-coated capillary (Minivette POCT) was bought from SARSTEDT Oy (Finland). For measuring plasma active GLP-1 level of mice, active GLP-1 ELISA kits (293-79301) were purchased from FUJIFILM Wako Pure Chemical Co. (Japan). Silicon wafers were purchased from Siegert Wafer GmbH (Germany), while ethanol (EtOH) was purchased from Alta Oy (Finland).

Fabrication of THCPsi Particles: Free-standing PSI films were fabricated by electrochemically etching boron doped, \(p^+\)-type Si (100) wafers of 0.01–0.02 \(\Omega\) cm resistivity in a 1:1 (vol.) HF (38%)-EtOH electrolyte using 50 mA cm\(^{-2}\) current density. The etched layers were lifted off from the wafers by applying an abrupt electropolishing current. The obtained PSI films were ball-milled and dried sieved to approximately 25–75 μm particle size. Before surface stabilization, the PSI particles were rinsed with the HF-EtOH etchant to remove native oxides formed during the milling, leaving the material hydrogen terminated. The particles were then thermally hydrocarbonized using acetylene (\(C_2H_2\))\(^{[21]}\). Particles were placed in a quartz tube under a continuous \(N_2\) flow. A 1:1 (vol.) \(C_2H_2: N_2\) flow was established at room temperature for 15 min followed by a 15 min thermal treatment at 500 °C using the same gas mixture. After the treatment, the obtained THCPsi microparticles were cooled to room temperature under a \(N_2\) flow.

THCPsi Particle Characterization: Structural characterization of the THCPsi microparticles was done with XPS, sorption at −196 °C using a TriStar 3000 (Micromeritics Inc., USA). The specific surface area was calculated from the isotherm using the Brunauer-Emmett-Teller (BET) method, while the total pore volume was obtained from the total amount adsorbed at a relative pressure \(p/p_0 = 0.97\). The average pore diameter was estimated with the BET area and total pore volume by assuming the pore shape to be cylindrical. The success of the hydrocarbonization treatment was verified with FTIR spectrometry. The infrared spectra of the THCPsi microparticles were obtained using an Inoveni R spectrometer (Bruker Optics, Germany) equipped with a PA301 photoacoustic detector (Gasera Oy, Finland). The morphology of the particles was studied using a field-emission scanning electron microscope (Thermo Scientific Apreo S, Netherlands).

Loading an AL4 to THCPsi Particles and TG: The impregnation method was applied for loading an AL4 to the THCPsi particles.\(^{[33]}\) anAL4 was dissolved in methanol and mixed with pre-weighted THCPsi particles in a glass flask with a magnetic stirrer. The amount of anAL4/methanol solution was calculated based on the targeted loading degree of 20% (w/w). The glass tube was incubated for 1 h under the stirring condition at room temperature. Then, the loading solution was evaporated under nitrogen flow for 1 h, and the loading degree was analyzed by TG (NETZSCH TG 209 F1 Libra). The anAL4 loaded THCPsi or unloaded THCPsi powders were placed into an open alumina crucible and kept at 40 °C for 30 min to remove adsorbed water. The samples were then heated up to 700 °C with a heating rate of 20 °C min\(^{-1}\). The anAL4 loading degree was calculated from the mass loss in anAL4 loaded THCPsi sample between 40 and 650 °C.

In vitro Release of anAL4 from THCPsi Particles into the Simulated Gastrointestinal Fluid: The release kinetics of anAL4 loaded THCPsi particles were studied in following biorelevant media: FaSSGF, FaSSIF, and SCoF.\(^{[34][35]}\) The composition of each media is shown in Table S1, Supporting Information. 1 mg of anAL4 loaded THCPsi particles were incubated with 1 mL buffer solution in a water bath at 37 °C with orbital shaking at a speed of 100 rpm. The tubes were incubated for a total of 24 h in the above media as indicated: FaSSGF (pH 1.6) for 1 h, FaSSIF (pH 6.5) for 1 h, FaSSIF without bile components (blank FaSSIF, pH 7.5) for 2 h, and SCoF (pH 5.8) for 20 h. The media and incubation time represent the simulated stomach, proximal small intestine, distal small intestine, and colon, respectively.\(^{[46]}\) At predetermined time points, supernatants were collected by centrifugation and stored at −20 °C until anAL4 analysis. The loaded compounds in time released mixtures were washed with 1 mL hexane to extract the unreleased anAL4 from the particles. Collected hexane solutions were subsequently evaporated overnight under vacuum conditions. anAL4 concentrations in the supernatant and remaining in the particles were analyzed by HPLC (SCL-10Amp, Shimadzu, Japan). The samples were separated on a C18 column (Symmetry, 5.0 μm, 100 Å, 3.9 × 150 mm, UVISION Technologies Ltd., UK) with acetonitrile and 0.1% TFA in the ratio 87:13 at a flow rate of 1 mL min\(^{-1}\) and anAL4 was detected at \(λ = 208\) nm. The pH of FaSSGF and SCoF samples were adjusted to pH 7 with 2M NaOH before the analysis. To evaluate the release kinetics of anAL4 from THCPsi particles, the Korsmeyer-Peppas equation (1): \(M_t/M_\infty = k t^n + b (1)\) was applied, where \(M_t\) is the amount of released compounds in time \(t\), \(M_\infty\) is initial compounds amount, \(k\) is the kinetic constant, and \(n\) is diffusional exponent.\(^{[37]}\) The n of 0.5 indicates a diffusional square root of time-release, 0.5 < n < 1 indicates the non-Fickian transport, \(n = 1.0\) shows zero-order release kinetics.

In vitro CLP-1 Secretion Study with STC-1 Cells: Twenty milligram of empty and anAL4 loaded THCPsi particles were weighted in the cell culture inserts. The inserts were placed in the host plate, and 500 and 800 μL of Krebs-Ringer Bicarbonate buffer (KREBS, 118 mM NaCl, 4.7 mM KCl, 25 mM NaHCO\(_3\), 1.25 mM CaCl\(_2\), 1.2 mM MgSO\(_4\), and 1.2 mM KH\(_2\)PO\(_4\)) were added to the insert wells and host plate wells, respectively. The inserts were utilized for collecting samples without particles and to protect cultured cells from detachment which may happen due to physical inter-
action between particles and cells.[8] KREBS buffer and 50 μM αLA were used as controls. All the samples contained 0.1% ethanol to assist in dissolving αLA into the buffer. Plates were incubated for 2 h in a water bath with orbital shaking at a frequency of 100 S min\(^{-1}\) at 37 °C. After 2 h, buffers from the host plate wells were collected, and 600 μL was used for measuring GLP-1 secretion from STC-1 cells and the remaining buffer was stored at −20 °C to analyze the αLA concentration by HPLC. STC-1 cells were pre-seeded into a 24-well plate and incubated until 90% confluent.[13] The cells were washed with KREBS and incubated for 1 h with KREBS buffer for acclimatization. The collected sample solutions with 0.25% DPP4 inhibitor were added to the cells and incubated for 1 h. The inhibitor was used for avoiding the degradation of secreted GLP-1. The supernatants were collected and centrifuged at 12 000 rpm, for 5 min at 4 °C, and GLP-1 concentrations were measured by ELISA (Merck, Germany). The minimum detection limit was 2 pm, and the intra-assay variations and inter-assay variations were 7.4% and 8.0%, respectively.

Animals: The National Animal Experiment Board of Finland approved the animal experiments (ESAVI/10110/04.07/2017). The work was conducted in accordance with the guidelines set by the Finnish Act on Animal Experimentation, Statute of Animal Experimentation, Animal Protection Legislation (62/2006, 36/2006, and HE32/2005), European Union Directive 2010/63/EU, and European Union Commission Recommendation 2007/526/EC. Inbred male C57BL6/NCrl mice age 9–11 weeks were purchased from the Laboratory Animal Center, University of Oulu, Finland. Mice were housed in an environment-controlled room with a 12 h light rhythm, with lights on at 6 am. Mice were fed ad libitum with commercial rodent chow (Teklad Global Rodent Diet T. 2018C, Harlan Teklad, USA), and had free access to tap water throughout the experiment. The temperature was set at 21 ± 2 °C and humidity was 50 ± 2.5%.

Effect of αLA on the Food Intake in Mice and Dosage Information: To determine the effective dose of αLA on the food intake, several doses of αLA were tested in mice using PhenoMaster (PhenoMaster-system, TSE Systems GmbH, Germany).[15,39] PhenoMaster is an automated system for measuring food and water intake, and metabolic performance/energy expenditure as well as physical activity in home cages. Mice (body weight 27.2 ± 0.2 g, n = 8) were acclimatized in training cages to the experimental conditions with mock dosing and handling. After 1 week of acclimatization, mice were orally dosed with either treatment (αLA 100, 200, and 500 mg kg\(^{-1}\) body weight) or control (water) 1 h before lights off. All the solutions were prepared in MQ water containing 2% DMSO and the dose volume was 8 mL kg\(^{-1}\) body weight. The food intake, water intake, energy consumption, and activity of mice were measured for the next 48 h after dosing. The activity of mice was detected by infrared sensors, and the O\(_2\) consumption, CO\(_2\) production, and heat production (kcal h\(^{-1}\)) were determined by the circuit-measuring system. The study was conducted as a cross-over study design, with a 5 days washout period between treatments. The doses of αLA we used in this study (100–500 mg kg\(^{-1}\) body weight) were within the dose range of αLA treatment in previous several animal studies.[40] αLA 200 mg kg\(^{-1}\) was selected as the reference dose for further experiments, which is equal to 1.62 mg kg\(^{-1}\) in human equivalent dose (HED).[41] and the recommended dose of αLA is 1.6 g per day for man and 1.1 g per day for women by US National Academy of Medicine.[42] GLP-1 Secretion in Mice: In vivo GLP-1 secretion with αLA loaded particles were studied in mice (body weight 27.5 ± 0.25 g, n = 34). Sitagliptin 25 mg kg\(^{-1}\) (DPP4 inhibitor) was orally administered in 5 h fasted mice to prevent GLP-1 degradation.[43,44] After 30 min, basal (0 min) blood samples were collected and mice were administered with control (MQ water, n = 7), glucose (3 g kg\(^{-1}\), n = 6), αLA 200 mg kg\(^{-1}\) (n = 8), 19% αLA loaded THCPSi particles (given an equivalent amount of αLA 200 mg kg\(^{-1}\), n = 7), and empty THCPSi particles (n = 6) dissolved in MQ water containing 2% DMSO and 0.5% CMC. The saphenous blood samples (40 μL) were collected at 5, 15, 30, 60, 120, 180, 240, and 300 min after dosing. Blood samples were collected via EDTA capillaries in prechilled tubes containing DPP4 inhibitors (Merck). To collect the blood samples from a mouse at indicated time points, the experiment was divided into 2 weeks. In the first week, blood samples were collected from half of the indicated time points, and after 2 weeks of recovering time, samples were collected from another half of the time points. Sitagliptin (half-life < 2 h) was administered again to the mice for longer time point sampling, for 120 and 180 min (sitagliptin at 90 min) and for 240 and 300 min (sitagliptin at 210 min).[45] Plasma was separated by centrifuging blood samples at 4000 x g, at 4 °C for 8 min, and stored at −70 °C until analysis. The plasma active GLP-1 levels were measured by an ELISA kit (FUJIFILM Wako Pure Chemical Co., Japan). The assay range was 0.615–150 pM, and both intra-assay and inter-assay variations were less than 10%. In addition, blood glucose levels were measured up to 60 min using glucose strips FreeStyle Freedom Lite (University Pharmacy OY, Finland).

Acute Effect of αLA Loaded THCPSi Particles on the Food Intake in Mice: THCPSi particles were successively loaded αLA with a loading degree of 19% for the food intake study in mice using PhenoMaster as described above. Briefly, mice (body weight 27.5 ± 0.4 g, n = 8) were orally administered with either αLA loaded or unloaded THCPSi particles along with positive (αLA 200 mg kg\(^{-1}\)) and negative controls (MQ water) 1 h before lights off. All samples were dissolved in MQ water containing 2% DMSO and 0.5% CMC. The study was performed as a cross-over study design, with a 5 days washout period between treatments.

Statistical Analysis: In vitro experiments were analyzed for statistical significance between the groups using one-way ANOVA followed by Dunnett’s multiple comparisons test. The multi-group comparisons of results from in vivo studies of 24 h food intake and metabolic parameters were performed by two-way ANOVA followed by Dunnett’s multiple comparisons test, and blood glucose, and plasma active GLP-1 were performed by mixed-effects model followed by Dunnett’s multiple comparisons test. The area under the curve (AUC) of 24 h food intake, 24 h water intake, and AUC of plasma GLP-1 were analyzed by one-way ANOVA followed by Dunnett’s or Tukey’s multiple comparisons test (Graph-Pad Prism 8, GraphPad Software Inc., CA, USA). Data are expressed as mean ± SEM. Differences were considered to be statistically significant when \(p < 0.05\).

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
The financial support from Jalmari and Rauha Ahokkaan Säätiö, Pohjois-Pohjanmaan Säätiö and Suomen Kulttuurirahastot (RK and GSR) are greatly acknowledged.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
Conceptualization, R.K., and K.H.H.; Methodology, R.K., G.S.R., J.R., and E.M.; Validation, R.K., G.S.R., J.R., and E.M.; Formal Analysis, R.K., J.R., and E.M.; Resources, Y.U., V.P.L., J.S. and K.H.H.; Writing – First Draft Preparation, R.K.; Writing – Review & Editing, R.K., M.K., J.R., E.M., G.S.R., K.H.H.; Supervision, K.H.H.

Data Availability Statement
The data supporting the findings of this study are available within the article and its supplementary information files and from the corresponding author upon reasonable request.

Keywords
α-Linolenic acid, enteroendocrine cells, food intake, GLP-1, mesoporous silicon particles
