Entrapping Digestive Enzymes with Engineered Mesoporous Silica Particles Reduces Metabolic Risk Factors in Humans

Erik R. Waara, Muhammad N. Iqbal, Ghislaine Robert-Nicoud, Boubacar Benziane, Helen Vallhov, Agata M. Wasik, Maria Lindgren, Emilia Hagman, Mia Rinde, Natalia Kupferschmidt, Roger Berlin, Eric V. Johnston, Pernilla Danielsson, and Tore Bengtsson*

Engineered mesoporous silica particles (MSP) are thermally and chemically stable porous materials composed of pure silica and have attracted attention for their potential biomedical applications. Oral intake of engineered MSP is shown to reduce body weight and adipose tissue in mice. Here, clinical data from a first-in-humans study in ten healthy individuals with obesity are reported, demonstrating a reduction in glycated hemoglobin (HbA1c) and low-density lipoprotein cholesterol, which are well-established metabolic and cardiovascular risk factors. In vitro investigations demonstrate sequestration of pancreatic α-amylase and lipase in an MSP pore-size dependent manner. Subsequent ex vivo experiments in conditions mimicking intestinal conditions and in vivo experiments in mice show a decrease in enzyme activity upon exposure to the engineered MSP, presumably by the same mechanism. Therefore, it is suggested that tailored MSP act by lowering the digestive enzyme availability in the small intestine, resulting in decreased digestion of macronutrient and leading to reduced caloric uptake. This novel MSP based mechanism-of-action, combined with its excellent safety in man, makes it a promising future agent for prevention and treatment of metabolic diseases.

1. Introduction

Synthetic mesoporous silica particles (MSP) have attracted attention for their potential biomedical applications such as the loading of poorly soluble drugs for the enhancement of drug bioavailability and for the delivery of bioactive factors in tissue engineering.[1,2] The controllable physical characteristics that make MSP interesting are the ordered porosity and pore dimensions, which give them high surface areas and large pore volumes, and different particle shapes and sizes.[3] In addition, MSP are thermally and chemically stable materials with high bio-compatibility. They are composed of amorphous silicon dioxide, which is an approved food additive (E551 under European Union regulations) and generally recognized as safe by the U.S. Food and Drug Administration (FDA). Silicon dioxide is also widely used as an excipient in the pharmaceutical industry and is described in the European Pharmacopoeia.[4]

Metabolic syndrome refers to the co-existence of a handful of known cardiovascular risk factors, including obesity, type 2 diabetes (T2D), dyslipidemia, and hypertension.[5] These conditions are interrelated and share underlying mediators, mechanisms, and pathways. Obesity is a condition in which fat accumulates in the body to a point where it has adverse effects on overall health, and it is also a risk factor for a number of other chronic diseases.[6] In 2016, the World Health Organization estimated that ≈13% of the world’s adult population had obesity, posing immense consequences on public health related efforts and costs.[7] T2D is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects
in insulin secretion, insulin action, or both.\cite{8,9} The International Diabetes Federation estimated that 425 million adults were living with diabetes worldwide in 2017.\cite{10} An important biomarker of hyperglycemia is HbA1c, a hemoglobin glycation marker found on red blood cells.\cite{11} The levels of HbA1c reflects the average blood plasma glucose levels over 8–12 weeks.\cite{12}

Another common comorbidity factor in individuals with obesity and T2D is dyslipidemia, defined as abnormal blood levels of lipids. In particular, elevated low-density lipoprotein (LDL)-cholesterol is linked to increased risk of cardiovascular disease and mortality.\cite{13}

Lifestyle changes such as exercise and diet are effective first in line interventions for people with metabolic syndrome but adherence is low.\cite{14} An interesting therapeutic strategy is to prevent the gastrointestinal (GI) absorption of lipids or carbohydrates after food intake, by affecting digestive enzymes such as lipase and α-amylase, which are critical for the digestion of lipids and complex carbohydrates, respectively. This is the mechanism-of-action of the enzyme inhibitors Orlistat (lipase inhibitor) and Acarbose (α-amylase inhibitor) that have demonstrated the efficacy of this therapeutic approach, but these drugs are associated with undesired side-effects.\cite{15,16} Several other drugs for metabolic disorders have also been tested, but so far, there are no effective treatments without potential severe side-effects.\cite{17,18} Finding safe and efficacious treatments is therefore desirable.

Several studies indicate that different forms of silicon dioxide affect metabolic parameters such as blood lipids and weight.\cite{19-22} Thus, another potential biomedical application of MSP could be in the treatment of the metabolic syndrome, including obesity and T2D. In the present study, we report the effects of oral intake of MSP on metabolic biomarkers in a first-in-man study in ten healthy individuals with obesity. We also report on preclinical in vitro, ex vivo, and in vivo experiments aiming to elucidate the mechanism of action of these particles.

2. Results

2.1. Material Characterization

Nitrogen sorption analysis was used to characterize the calcined silica particles and results are presented in Table 1. The Brunauer–Emmett–Teller (BET) surface area, micropore area, and pore volumes of all batches are in accordance with previous reports of these types of silica particles.\cite{21,24}

The nitrogen sorption isotherms show curves typical of mesoporous silica materials. All batches, except SM0002, exhibit a clear hysteresis loop and can be classified as type IV according to the International Union of Pure and Applied Chemistry nomenclature (Figure 1A).\cite{25}

It is generally recognized that there is a connection between the shape of the hysteresis loop and some properties of the material such as pore geometry, pore connectivity, and pore size distribution. All batches, except SM0002, are clearly type H1 hysteresis loops (Figure 1A), which is associated with well-defined cylindrical pores.\cite{26} Hysteresis in the desorption branch, typical of Type IV isotherms, is absent from SM0002 due to the small size of the mesopores, as previously described for this type of MSP.\cite{24} All silica batches have a relatively narrow pore size distribution (Figure 1B). Scanning electron microscope (SEM) images of SM0023, SM0026, SM0071, SM0077, and SM0076 show large agglomerates of several micrometers composed of rod-shaped silica particles of length varying from 1 to 3 µm while batch SM0002 is composed of larger hollow spherical particles (Figure 1C). The final morphology of MSP is determined by the self-assembly of the template and rate of particle formation. The morphology of SM0002 is very different to the morphology of the other materials because SM0002 was prepared with a different template. Except for SM0002, the template was the same for all MSP presented here, but SM0071 was formed at 50 °C while the other materials were formed at 40 °C. Because of the higher temperature, the particles grew faster in the longitudinal axis, forming longer, thinner particles that can bend and form loops. The other particles have similar rod-shaped morphologies but of varying length and diameters. Even at the same reaction temperature, a lower tetraethyl orthosilicate (TEOS)/HCl ratio could have influenced reaction rates, causing the formation of longer, thinner particles in SM0076 and SM0077 (TEOS: HCl = 1:7) compared to SM0023 and SM0026 (TEOS: HCl = 1:6).

Transmission electron images of batches with representative pore sizes (SM0002-3.3, SM0077-5.7, SM0023-9.6, and SM0076-11.8 nm) are presented in Figure S1 in the Supporting Information. In addition, X-ray diffraction (XRD) pattern of SM0023 (Figure S1, Supporting Information) is presented in the Supporting Information and confirms that SM0023 has a 2D hexagonal pore structure, as expected for this class of silica particle.\cite{23} SM0097 and SM0056 were prepared similarly to batch SM0023, with the exceptions that one portion of SM0097 was kept uncalcined in order to have particles with templating

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**Table 1. Characterization data of calcined silica materials.**

| Batch   | Specific surface area [m² g⁻¹] | Micropore area [m² g⁻¹] | Total pore volume [cm³ g⁻¹] | Mean pore size [nm] |
|---------|--------------------------------|-------------------------|-----------------------------|---------------------|
| SM0002  | 541                            | 0                       | 0.42                        | 3.3                 |
| SM0023  | 684                            | 247                     | 0.88                        | 9.6                 |
| SM0026  | 752                            | 344                     | 0.72                        | 7.9                 |
| SM0056  | 882                            | 312                     | 1.16                        | 11.0                |
| SM0071  | 719                            | 346                     | 0.57                        | 7.1                 |
| SM0076  | 815                            | 226                     | 1.25                        | 11.8                |
| SM0077  | 696                            | 301                     | 0.52                        | 5.7                 |
| SM0097  | 876                            | 373                     | 1.06                        | 9.7                 |

\(\frac{a}{\text{plot}} \quad \frac{b}{\text{DFT cylindrical fit.}}\)
polymer remaining inside the pores, to test in an enzyme activity experiment, and SM0056 was labeled with magnetic particles (SM0056-mag), to test in an in vivo experiment. Calcined SM0097 and SM0023 have similar nitrogen adsorption isotherm, pore size distributions, and particle morphology (data not shown). SM0056-mag has a pore size of 14 nm but is otherwise similar to SM0023 with a clear Type-H1 hysteresis loop in its adsorption–desorption isotherm, a narrow pore size distribution, and rod-shaped particles morphology (Figure S2, Supporting Information). SM0002 also labeled with magnetic particles and the resulting material (SM0002-mag) had similar characteristics and pore size as SM0002 (Figure S2, Supporting Information).

2.2. Effects on Metabolic Risk Factors in Human Volunteers

The main aim of the first-in-humans trial was to investigate safety and tolerability of oral MSP treatment. Only mild and tolerable adverse events were observed and the results did not raise any safety concerns; the detailed clinical protocol and the core dataset generated in this study are described in a recently submitted report (E. Hagman, A. Elimam, N. Kupferschmidt, K. Ekbom, S. Rössner, M. N. Iqbal, E. V. Johnston, M. Lindgren, T. Bengtsson, P. Danielsson, submitted manuscript).

Notable were findings related to the reduction in metabolic risk factors. Despite the small sample size, in the ten sub-
jects with obesity there was a significant decrease in long-term plasma glucose concentration, measured as glycated hemoglobin (HbA1c) from 34.7 to 33.0 mmol mol\(^{-1}\) after 12 weeks of treatment (Figure 2A,B). In addition, these same subjects showed a statistically significant lowering in plasma LDL cholesterol after 12 week treatment, from 2.6 to 2.2 mmol L\(^{-1}\) (Figure 2C,D) while their plasma high-density lipoprotein (HDL) cholesterol was statistically unchanged (Figure S3, Supporting Information). As a result, the LDL/HDL ratio of the subjects with obesity also decreased significantly from 2.2 to 1.9 (Figure S3, Supporting Information). The ten normal weight volunteers, followed for 3 weeks, showed a statistically significant decrease in plasma LDL cholesterol (from a mean 2.7 to 2.4 mmol L\(^{-1}\)) following 3 weeks of treatment with MSP (Figure S3, Supporting Information).

2.3. Pore-Size Dependent MSP Entrapment of Digestive Enzymes

In order to investigate if gastric enzymes are entrapped inside the pores, we performed a series of in vitro experiments utilizing purified enzyme solutions of porcine pancreatic origin. Initial in vitro experiments were conducted after incubating pure porcine \(\alpha\)-amylase with particles having a pore size of 9.7 nm (hereinafter referred to as “open” particles, batch SM0097). In parallel pure porcine \(\alpha\)-amylase was incubated with the same noncalcined particles where the polymer (P123) remains inside the pores, blocking the particle pores (here referred to as “closed” particles). We observed that the “open” particles decrease \(\alpha\)-amylase activity in an MSP concentration dependent manner, whereas the “closed” particles did not (Figure 3A). In a second experiment, we performed the same \(\alpha\)-amylase activity assay on a panel of in-house generated silica particles with pore sizes ranging from 3.3 to 11.8 nm (Figure 3B). The \(\alpha\)-amylase enzyme activity was lowered by particles with pores sizes ranging from 8.0 to 11.8 nm, with particles of 8.0 nm pore size only affecting \(\alpha\)-amylase activity at higher MSP concentration. The \(\alpha\)-amylase activity was unaffected when exposed to MSP with pore size of 7.1 nm and below, independent of the particle concentration (Figure 3B). This illustrates that pore size is an important factor affecting the enzyme activity in vitro.

In a follow-up experiment the silica particles were again incubated with pure \(\alpha\)-amylase and the protein concentration in the solution was measured using a bicinchoninic acid (BCA) assay. The protein concentration in solution after removing the silica particles by centrifugation was decreased by more than 80% when incubating with 1.2 mg mL\(^{-1}\) of SM0023 (pore size of 9.6 nm) (Figure 4A). However, incubation with silica particles with a smaller pore size (SM0002, pore size of 3.3 nm) did not change the protein concentration in the solution. Next, we assessed the effect on enzyme activity and observed that SM0023 was able to decrease the \(\alpha\)-amylase activity by 70%, whereas...
SM0002 did not alter the α-amylase activities (Figure 4C). As a continuation, we assessed the interaction with pure porcine pancreatic lipase, a key digestive enzyme involved in lipid digestion. Similarly, silica particles with a pore size of 9.6 nm decreased the lipase protein concentration by 80% and lipase activity by 65% (Figure 4B,D). The particles with a 3.3 nm pore size decreased the lipase protein concentration and lipase activity by 30% each (Figure 4B,D). In addition, in vitro experiments with pure α-amylase reveal substantial entrapment already after 30 min incubation, with a saturated adsorption after 3 h of approximately 125 µg α-amylase mg⁻¹ MSP (Figure S4A, Supporting Information). In comparison, the adsorption of lipase was clearly faster with an almost saturated entrapment already at 10 min and a maximum entrapment of ≈250 µg lipase mg⁻¹ MSP at 30 min (Figure S4B, Supporting Information).

2.4. Enzyme Entrapment in Complex Biological Fluids

To mimic a physiological environment, we evaluated the effects of SM0002 on both lipase and α-amylase in a solution of porcine pancreatin, a more complex mixture of enzymes produced by the exocrine cells of the porcine pancreas. As assessed by western blot analysis, α-amylase and lipase concentrations were decreased after incubation with SM0002 at 1.25 and 0.31 mg mL⁻¹, respectively (Figure 4E,F). Again, both α-amylase and lipase enzyme activities were dramatically lowered (>80%) after incubation with SM0002 while the silica with small pore size (SM0002) did not affect the enzymes concentration nor the enzymes activity (Figure 4G,H). As loading controls, we used the principle of total protein normalization and the total protein content in the supernatants remains unaffected, regardless of how much MSP the sample has been exposed to (Figure S5, Supporting Information).

We then investigated the MSP pore-size dependent effect on α–amylase in murine intestinal fluid, another physiologically relevant environment (Figure 5). The results show that SM0002 at 10 mg mL⁻¹ reduced the α-amylase activity below 20% of the activity without MSP (Figure 5B). In comparison, batch SM0077, with a mean pore size of 5.7 nm, did not alter the activity (Figure 5B). The decrease in α-amylase activity was marked by a reduction in the amount of α-amylase in supernatant as measured by western blot analysis (Figure 5C). As loading controls, we used the principle of total protein normalization and the total protein content in the supernatants remains unaffected, regardless of how much MSP the sample has been exposed to (Figure S5, Supporting Information). As expected, analysis of the precipitated silicas revealed that a larger amount of α-amylase was retained by SM0023 as compared to SM0077 (Figure 5D).

In a follow-up in vivo experiment, mice were gavaged with different doses of magnetic MSP and 40 min later the silica was recovered from the small intestine of the animals (Figure 6). The isolated MSP with a pore size of 14 nm retained an average of 2 µg α-amylase at a dose of 400 mg kg⁻¹ (Figure 6B). Inoculating MSP with pores of 3.6 nm at the same dose resulted in minute amounts of α-amylase being recovered together with the silica. Lower α-amylase amounts were recovered from the two groups of animals receiving the lower MSP doses (100 and 10 mg kg⁻¹), showing a dose-dependent α-amylase entrapment. To control for the MSP exposure, we compared the amount of MSP being gavaged and retrieved and observed a similar recovery from the SM0002-mag and SM0056-mag treated animals (Figure 6C).

3. Discussion

In our recent clinical study, we concluded that oral intake of MSP is safe and well tolerated and the details of these findings are reported elsewhere (E. Hagman, A. Elimam, N. Kupferschmidt, K. Ekbom, S. Rössner, M. N. Iqbal, E. V. Johnston, M. Lindgren, T. Bengtsson, P. Danielsson, submitted manuscript). In the same study we observed improved glucose and lipid homoeostasis, data
Figure 4. Engineered mesoporous silica sequester α-amylase and lipase in vitro. MSP batch SM0023 with a pore-size of 9.6 nm and MSP batch SM0002 with a smaller pore-size (3.3 nm) were incubated with pure porcine extracts of (A) α-amylase or (B) porcine lipase followed by centrifugation and quantification of the supernatant protein concentration using a commercial BCA assay kit. (C) The α-amylase enzymatic activity and (D) the lipase enzyme activity were also measured in the supernatant. MSP batches SM0023 and SM0002 were also incubated with commercial porcine pancreatin. The entrapment of the enzymes was monitored using a Western blot protocol utilizing (E) anti-α-amylase and (F) antilipase monoclonal antibodies. The corresponding (G) α-amylase and (H) lipase enzyme activities in the pancreatic fluids were assessed by adding the corresponding substrates. Each experiment has been repeated at least twice, with one representative dataset shown here. The data are expressed as a percentage, normalized to the enzyme alone (white bar) tubes. SM0002 and SM0023 are tested here in duplicate tubes and data are shown as mean ± standard deviation. The student’s T-test was used to compare untreated versus MSP treated values. ns = not significant, ** p < 0.01, *** p < 0.001.

reported herein (Figure 2) together with preclinical findings supporting a postulated mechanism of action. These clinical observations, together with preclinical data on reduced weight gain in mice,[28] led us to envisage a novel therapeutic principle. We hypothesized that oral intake of MSP with a defined pore size would sequester digestive enzymes in the GI tract. This would result in reduced food digestion, reduced energy uptake, and improvement of metabolic parameters.

Here we show that treating human obese subjects for 12 weeks with MSP lead to an average 1.7 mmol mol⁻¹ decrease in the long-term blood glucose marker HbA1c. It is important to keep in mind that the study was designed to address
Figure 5. Engineered mesoporous silica adsorb murine pancreatic \( \alpha \)-amylase ex vivo. A) A C57BL/6J mouse was induced to secrete intestinal fluid by oral gavage, 40 min later the animal was sacrificed, and the fluid was isolated. The intestinal fluid was exposed to MSP batch SM0023 and batch SM0077 with small pores \((5.7 \text{ nm})\) ex vivo, followed by centrifugation of the sample. The murine supernatants were B) incubated with starch to assess the enzyme activity and C) used to detect \( \alpha \)-amylase in the solution by an anti-\( \alpha \)-amylase specific western blot protocol. D) The MSP pellet generated in (A) was washed thoroughly, boiled, and analyzed by western blot. The experiment has been repeated two times with similar results. The data points shown in (B) are duplicate well means in a 96-well plate and the variation is shown as standard deviation.

safety and not to study effects on metabolic markers. The subjects with obesity studied herein had normal baseline HbA1c levels and did not have T2D. Thus the results herein cannot be directly compared to studies with higher HbA1c baseline levels, as this is known to result in more pronounced effects on HbA1c.\(^{[29]}\) Still, it is interesting that studies with the most commonly used antiobesity drug Orlistat (lipase inhibitor) suggest an \( \approx 4 \text{ mmol mol}^{-1} \) reduction in HbA1c in subjects that have both obesity and T2D.\(^{[30]}\) Moreover, diabetes prevention studies have looked at the effect of Metformin, a common T2D drug, on HbA1c in populations similar to the one studied here and have found that a reduction of 1.0–1.6 mmol mol\(^{-1}\) significantly reduced the risk of developing diabetes.\(^{[31,32]}\) Furthermore, we anticipate that in a population with higher basal HbA1c level such as subjects with T2D, the effect of MSP treatment would be greater.

Even with the small number of study subjects reported here, we observed a statistically significant reduction in LDL cholesterol already after 3 weeks, with a further decrease after 12 weeks (average decrease of 0.4 mmol L\(^{-1}\)). A reasonable comparison could be with statins, the most common LDL lowering drugs. In a large meta-analysis, statins decrease LDL on average by 1.09 mmol L\(^{-1}\) after one year of treatment.\(^{[33]}\) Again, the exact comparison is uneven, since our subjects were only treated for 3 months and had normal LDL baseline levels. Another comparison could be with large numbers of Orlistat treated (lipase inhibitor) individuals with obesity, showing LDL cholesterol reductions in the 0.21–0.47 mmol L\(^{-1}\) range.\(^{[30]}\)

There are likely a number of factors influencing the MSP-mediated effects in humans, such as dietary pattern, meal composition, lifestyle, physiological factors, and pathological conditions. The number of subjects treated in this study is small, therefore it can only be speculated as to why there are individual differences in the MSP mediated responses in HbA1c and LDL. The individual variation in HbA1c/LDL reduction seen here could be explained by changes in gross dietary intake, as it is not controlled for here. In addition, one could imagine that individual variation in HbA1c related genetics and/or the copy numbers of the Amylase gene or genetic differences in lipid carrying molecules such as Apolipoproteins could result in different treatment outcomes.\(^{[34–36]}\) Questions concerning the retention and clearance of MSP during the GI passage are also of relevance. Due the insolubility of silica it will not be readily adsorbed nor broken down in the gastrointestinal tract, but instead most likely excreted in the feces.\(^{[37]}\) Finally, we believe that the MSP mediated effects on HbA1c and LDL observed in this study show the potential to be clinically meaningful and merit further clinical investigations, including a more detailed analysis of its fate during and after the GI passage.
In a series of preclinical experiments, we tested our enzyme entrapment hypothesis and clearly demonstrate that specifically engineered mesoporous silica particles entrap pancreatic α-amylase and lipase, decreasing the concentration of enzymes. The effect of our particle is pore-size dependent and our data illustrate that a pore-size range of 8.0–11.8 nm is optimal for digestive enzyme entrapment in vitro. This pore-size range is in agreement with previous reports of lipase immobilization on MSP for industrial purposes.\textsuperscript{[38,39]} In addition, the enzyme entrapment kinetics estimated here is similar to what has been observed by others.\textsuperscript{[40]} To illustrate that the enzymes are entrapped inside the pores we performed an experiment where the pores were blocked, and the α-amylase entrapment did not occur. The entrapment principle is also supported by preliminary in vitro data gathered after exposing MSP (batch SM0023) to α-amylase in solution for 30 min followed by a wash-out step over 2 h, which does not result in any detectable α-amylase being release into the solution over the 2 h studied (data not shown). The enzyme entrapment principle also holds true in porcine pancreatic fluid and in murine intestinal fluids ex vivo, demonstrating the entrapment in a more complex biological fluid, containing a multitude of different biomolecules.\textsuperscript{[41]} Further, in vivo experiments demonstrated that magnetic MSP recovered from the small intestine of mice entrapped α-amylase in a pore size and dose dependent manner. This experiment was not designed to determine the effective dose to be translated to a clinical protocol. However, the mouse data suggest that a one-time dose of 400 mg kg\textsuperscript{-1} has an effect on α-amylase adsorption greater than that of treatment with control silica. Using the allometric scaling from mouse-to-man suggest that a human dose of 2.3 g (0.4 g kg\textsuperscript{-1}/factor 12.3) x 70 kg should have an effect on α-amylase adsorption in humans.

There is a well-established relationship between the MSP pore size and the dimensions of the protein that can be entrapped.\textsuperscript{[42]} Given that α-amylase and lipase have similar dimensions (≈5 nm × 7 nm × 13 nm) as the MSP pores,\textsuperscript{[43]} it is most likely that the actual molecular size of the enzymes is essential for the entrapment. Once the enzymes have passively diffused into the MSP it is probable that they do not easily exit the structure.\textsuperscript{[44]} However, it is plausible that lipase partially binds to the external surface of MSP particles, in line with the observation that batch SM0002 (with pores too small for the enzyme to enter) still decreased ≈30% of the lipase activity in vitro (Figure 4B,D). It is known from the literature that factors such as surface charge, buffer ionic strength and pH, together with pore size in relation to enzyme size, will determine how difficult it will be for the enzyme to exit the MSP structure.\textsuperscript{[44]} All entrapment factors are likely to be of clinical importance and we are therefore planning more in-depth investigations into the MSP mediated retention of gastrointestinal enzymes. Furthermore, it is likely that other material characteristics such as the arrangement of pore network, microporosity, surface charge, and hydrophilicity could affect the interaction with the enzymes.\textsuperscript{[45–47]} The pH of the enzyme solution is also a known important parameter that will
Figure 7. The gastrointestinal enzyme entrapment principle—proposed mechanism of action for engineered mesoporous silica. Once food has reached the duodenum, its complex carbohydrates and triglycerides are subjects to enzymatic degradation by pancreatic α-amylase and lipase. Upon oral MSP intake, a portion of the gastric enzymes are entrapped inside the particles whereas complex carbohydrates and triglycerides are too large to enter the pores. The enzyme concentration and thereby enzymatic activity decreases in the free intestinal fluid outside the MSP. Consequently, the amount of digested short carbohydrates and lipids is decreased and less micronutrients pass across the epithelial into the blood circulation.

Influence of enzymes adsorption. In theory, increased entrapment could possibly be achieved by more sophisticated chemical modifications; however, this would then move away from a material consisting of pure silica, which would alter the safety profile. In addition, we cannot rule out that other interactions might also be involved, such as adsorption of other GI enzymes, lipids, carbohydrates, and bile acids. We are planning to perform more extensive studies to decipher in greater detail what other biomolecules are being entrapped by the MSP.

Interestingly, other preclinical data generated by us and collaborators corroborate the findings reported herein. Experiments in a rat postprandial model suggest that MSP entrap lipase resulting in reduced blood lipid levels (K. L May, A. J Clulow, T. Bengtsson, E. V. Johnston, B. J. Boyd, manuscript in preparation). Another series of experiments demonstrated an attenuated weight gain in mice treated with engineered MSP. The study showed reduced food efficiency in the MSP fed mice, meaning that a smaller percentage of the total food intake was metabolized into body fat mass. This strongly indicates an effect on food digestion. Furthermore, an earlier preclinical study showed a significant weight loss (up to 5%) in mice treated with MSP, an effect linked to reduction in adipose tissue, again pointing at an altered lipid metabolism. Future preclinical and clinical studies could further explore the MSP mediated mechanism of action.

4. Conclusion

Here, MSP have been shown to clinically reduce HbA1c and LDL-cholesterol in a first-in-humans study with ten healthy individuals with obesity. Furthermore, preclinical investigations demonstrated MSP sequestration of pancreatic α-amylase and lipase in a pore-size dependent manner. Based on the results presented herein, we propose a mode-of-action model in which MSP entrap α-amylase and lipase, hindering and delaying the digestion of food and the absorption of nutrients in the human GI tract (Figure 7). This novel mode-of-action, together with an excellent safety profile, makes MSP an enticing candidate for prevention and/or treatment of metabolic diseases. A follow-up clinical trial is ongoing in 40 patients with prediabetes or T2D (clinicaltrials.org, NCT03823027).

5. Experimental Section

Preparation of Mesoporous Silica Particles: Prototype particles were synthesized by a modified method as reported previously. All the chemicals used in the synthesis were purchased from Sigma-Aldrich. A panel of batches with varying pore size were prepared by varying the synthetic method. In brief, a meso-structure templating agent (P123, a triblock copolymer with average molecular weight = 5800 g mol⁻¹, ...
PEO₉₀PPO₇₀PEO₉₀ was dissolved in aqueous hydrochloric acid, with acid concentration equivalent to 1.6 M. Complete dissolution of P123 was followed by addition of TEOS under vigorous stirring at 40 °C. The final molar ratio of P123: TEOS in the solution was 0.02:1.00 and the molar ratio of TEOS: HCl: H₂O varied from 1.5:221 to 1:2752. Batch SM0007 was prepared by heating the reaction to 50 °C under static conditions for 24 h, with no further hydrothermal treatment. For the remaining batches (SM0002, SM0026, SM0056, SM0076, SM0077, and SM0097), the synthesis was kept static at 40 °C for 20 h and further hydrothermally treated for 1.3 h at 85 °C (SM0077), 20 h at 85 °C (SM0026), 10 h at 100 °C (SM0076 and SM0097), or 20 h at 100 °C (SM0032 and SM0056). Following hydrothermal treatment, the material was filtered, washed, and dried. Batch SM0076 was further treated by heating a suspension of material (220 mg mL⁻¹) in an aqueous solution of P123 (31 mg mL⁻¹) at 185 °C in an autoclave for 72 h. Batch SM0056 was labeled with magnetic nanoparticles (SM0056-mag) by adding a suspension of SM0056 in water (120 mg mL⁻¹), prepared by sonication for 15 min at a 40% amplitude using an ultrasonicator equipped with a 3 mm probe (Sonics Vibra-Cell), to an equal volume of a solution of iron oxide magnetic nanoparticles (8 mg mL⁻¹) prepared separately according to a modified method previously reported. After 5 min stirring, the pH was adjusted to 7.0–7.2 using concentrated NH₄OH and acetic acid aqueous solutions and the silica/magnetic particles suspension was left at room temperature overnight under stirring. MSP particles were recovered by centrifugation, washed, and dried. A silica comparator with smaller pore size was made according to modified protocols from the literature. It is named herein as SM0002. A portion of SM0002 was also labeled with magnetic nanoparticles by the method described above and was named SM0022-mag. All batches were finally subjected to calcination (550 °C in air) to remove the organic template and generate the porous material. Prior to in vitro and in vivo experiments, the silica material was dried overnight at 120 °C, suspended in water at a concentration of 20 mg mL⁻¹ followed by sonication for 2 × 3 min at a 40% amplitude using an ultrasonicator equipped with a 2 mm microphone (Sonics Vibra-Cell).

Material Characterization: Nitrogen adsorption/desorption isotherms were measured at liquid nitrogen temperature (−196 °C) using a Tristar II volumetric adsorption analyzer (Micromeritics Instrument Corp.). Before being measured, the samples were outgassed for a minimum of 15 h at 150 °C at 0.03 mBar. Data analysis was performed with the Tristar II 3020 software (Micromeritics Instrument Corp.). BET surface area was calculated from adsorption isotherm at a relative pressure (p/p°) of 0.2. Micropores surface area was calculated using t-plot method from the desorption branch of the isotherm within the 0.5−1 nm thickness. Total pore volume was recorded at a relative pressure (p/p°) of 0.99. Pore size distribution curves were obtained by applying the density functional theory (DFT) method to the adsorption isotherm assuming a cylindrical pore model. The morphology of the particles was characterized by SEM on a JEOL JSM-7401F equipped with a Schottky-type field emission gun as electron source operating at an accelerating voltage of 1 kV without any sample coating. Pore structure was characterized by low-angle XRD on a powder PANalytical X’Pert Pro diffractometer (PANalytical), equipped with Cu Kα radiation source, operated at 45 kV and 40 mA, scanning at low angle from 0° to 8° 2θ with a 0.02° step size under transmission mode, with no background. Pore structure was also observed by Transmission Electron Microscopy using a JEOL JEM-2100F instrument (JEOL Ltd.) equipped with LaB6 emission gun.

First-In-Humans Clinical Trial: A first-in-humans study was designed with the aim to explore the safety and tolerability of daily oral MSP ingestion over 12 weeks, as reported elsewhere (E. Hagman, A. Elimam, N. Kupferschmidt, K. Ekborn, S. Rössner, M. N. Iqbal, E. V. Johnston, M. Lindgren, T. Bengtsson, P. Daniellson, submitted manuscript). In addition, the metabolic biomarkers HbA1c, LDL, and HDL were monitored and the results are reported here. The silica particles used in the first-in-humans trial were synthesized according to food grade regulations. The mean pore size of the studied material was in the range 7–12 nm. Details referring to material characterization are reported by Hagman and co-workers. In brief, 20 male volunteers were included (18–35 years old); in which ten participants were of normal weight with body mass indexes (BMI) in the range 20.0–25.0 kg m⁻² and ten participants had obesity (BMI 30.0–45.0 kg m⁻²). All the volunteers were treated with a final oral MSP dose of 9 g d⁻¹ (3 g with each meal), a dose based on two criteria. First, the estimated daily dose of silicon dioxide recognized as safe is 10–30 g according to the FDA, equivalent of 2% silicon dioxide in normal daily food intake of 500–1500 g [25]. Second, the NOAEL (no-observed-adverse-effect level) by mesoporous silica in rodent models was determined to be 2.5 g kg⁻¹ d⁻¹ as concluded in the European Food Safety Agency, which corresponds to a human equivalent life-long dose of 24 g d⁻¹ if using body surface area-based alometric scaling. Consequently, the daily dose of 9 g is below both the FDA criteria and the NOAEL based estimation. Informed consent was obtained and the trial was carried out in accordance with the Declaration of Helsinki. The study was approved by the Regional Ethical Review board, Stockholm, Sweden (2015/593-31) and registered in the Clinical Trials Registry (clinicaltrials.gov, NCT03667430).

Lipase Activity Assay: The ability of silica particles to sequester pancreatic lipase was studied by measuring a suspension of silica (0–10 mg mL⁻¹) with pure porcine lipase (LEE Biosolutions, cat. no. 400-10) or porcine pancreatin (Sigma-Aldrich, cat. no. P1625), both dissolved in 2 × Tris buffer at pH 5.4 (Trizma base, Sigma-Aldrich, cat no. T6066). Pure lipase was dissolved at a concentration of 0.660 mg mL⁻¹, and porcine pancreatin at a concentration of 3.2 mg mL⁻¹. Both solutions were centrifuged at 1500 rcf for 30 min at 4 °C. The supernatant was repeated with porcine pancreatin at 1000 rcf for 10 min at 4 °C. The supernatant was used as the source of enzyme and added to the silica. The mixture was incubated in a 96-well PCR plate (Bio-Rad, cat. no. MILL9601) at 37 °C for 30 min with vertical rotation (Harvard apparatus, cat no. 74-2302). The plate was centrifuged at 3000 relative centrifugal force (rcf) for 5 min and the supernatant was transferred to a new plate and incubated for another 30 min together with 1.5 mg mL⁻¹ soluble starch (Sigma-Aldrich, cat no. 33615), following the addition of 3,5-dinitrosalicylic acid (DNS) (Sigma-Aldrich, cat no. D0550) and heating at 95 °C for 7 min in a conventional PCR machine (Corbett Research, PC 960C). DNS changes color in the presence of reducing sugars and was detected at 540 nm using an absorbance reader (EnSpire, Perkin Elmer). This α-amylase assay protocol was developed in-house and adopted from published protocols. In parallel, a BCA assay kit was utilized to measure the protein concentration in the supernatants (QuanPro, Sigma-Aldrich cat no. QPCBA).

Western Blot Analysis: Porcine pancreatic supernatants, murine intestinal fluid samples, or isolated magnetic MSP were dissolved in Laemmli sample buffer (Thermo Fisher Scientific, cat no. B0007) and reduced agent (Thermo Fisher Scientific, cat no. B0009) and heated for 15 min at 65 °C. The amount of pancreatic supernatants and murine intestinal fluid loaded onto the sodium dodecyl sulfate-polyacrylamide gel.
electrophoresis (SDS-PAGE) gels were the same. As loading controls, the principle of total protein normalization was used by quantifying the amount of total protein in all samples using a BCA kit (QuantPro, Sigma-Aldrich cat no. QPBCA).[27] For the in vivo data (Figure 6) a five-point dilution series of porcine α-amylase (Sigma-Aldrich, cat no. A6255) was used as standard for quantifying the protein bands. Sample and standards were separated by SDS-PAGE using 4–12% gradient gels (Invitrogen, cat no. NW04125BOX). Following electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad, cat no. 170-4156). Membranes were blocked with a milk-free blocking buffer (Li-cor, cat no. 927-50000) for 1 h at room temperature and then incubated overnight at 4 °C with an antipancreatic α-amylase antibody (Abcam, cat no. ab21156) or an antipancreatic lipase antibody (Abcam, cat no. ab31154) in a mixture of tris-buffered saline and Tween20 (TBS-T) (10 × 10⁻⁴ M Tris, 100 × 10⁻⁴ M NaCl, and 0.5% Tween 20) containing 50% blocking buffer. Membranes were washed 3 × 10 min with TBS-T following incubation for 1 h at room temperature with a goat-antirabbit fluorescent conjugate (Li-cor, cat no. 925-32211) or goat-anti-duck-horseradish peroxidase (HRP) conjugate (Abcam, cat no. ab112773) in TBS-T containing 20% blocking buffer. Finally, the membranes were washed 3 × 10 min in TBS-T and 3 × 10 min in pure TBS. The lipase blots detected with HRP conjugated secondary antibody were developed using Amersham enhanced chemiluminescence prime Western blotting detection kit (Amersham, cat no. RPN2232). Immuno-reactive proteins were visualized at the appropriate wavelength by the Odyssey FC Imager (Li-cor).

Animal Studies and Gastrointestinal Sampling: Experiments were carried out in male C57BL/6 mice (16 weeks old; Scanbur Breeding Laboratories). The studies were approved by the animal ethics committee of the North Stockholm Region (reference no. N240/14). Mice had free access to chow diet (R70, Lactamin) and were allowed to drink water ad libitum. In order to obtain samples for the ex vivo experiments (Figure 5) oral gavage was performed on one animal with water at 10 mL kg⁻¹ to induce pancreatic juice secretion. In order to study the MSP mediated in vivo effects (Figure 6) 12 animals were fasted for 5 h following oral gavage with MSP batch SM0002-mag at 400 mg kg⁻¹ and MSP batch SM0056-mag at 400, 100, and 10 mg kg⁻¹ in four groups of three animals each. After 40 min the animals were sacrificed by CO₂. A 5 cm long section of the small intestine (10–15 cm below stomach) was removed and flushed with 0.4 mL cold PBS supplemented with 1% v/v antiprotease cocktail (Sigma-Aldrich, cat no. 4693159001). The ex vivo samples were stored immediately at −20 °C until analysis. The magnetic MSP from the in vivo samples were isolated using a microcentrifuge tube magnet (Invitrogen, cat no. 10723874) and the MSP was washed three times with PBS containing the antiprotease cocktail. The isolated magnetic MSP was dried at 100 °C overnight to remove residual water and weighed prior to storage at −20 °C until further analysis by western blot.

Statistical Analysis: The data are reported as mean ± standard deviation (SD). Sample size and data processing are reported for each dataset under the respective figure legend. Statistical significance comparing two groups was assessed by Student’s t-test. Differences among the means were accepted as p < 0.05. Statistical analysis was performed using the Prism GraphPad software.

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

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Conflict of Interest
E.R.W., M.N.I., G.R.-N., B.B., H.V., A.M.W., M.L., and E.V.J. are or have been employees of Sigrid Therapeutics AB. T.B. and R.B. hold shares in Sigrid Therapeutics AB.

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