Mesoporous silica with precisely controlled pores reduces food efficiency and suppresses weight gain in mice

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Aim: Obesity is a risk factor for cardiovascular disease and diabetes. We aimed to elucidate the effects of distinct mesoporous silica particles (MSPs) supplemented in food on metabolic parameters in obesity. **Materials & methods:** MSPs with precisely controlled pore size were synthesized, characterized and compared with a control in a C57Bl/6 mouse diet-induced obesity model, studying weight, adiposity, metabolic regulation and food efficiency. **Results:** The most effective MSPs reduced adipose tissue formation to 6.5 ± 0.5 g compared with 9.4 ± 1.2 g, leptin levels nearly halved from 32.8 ± 7.4 to 16.9 ± 1.9 ng/ml and a 33% reduction of food efficiency. Control MSP showed no effects. **Conclusion:** Results demonstrate potential of distinct MSPs to improve metabolic risk factors. Further studies investigating mechanism of action and confirming human safety are needed.

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Obesity is a chronic, debilitating condition that can lead to devastating health effects. Today, 13% of the world's adult population is obese [1], defined as having a body mass index above 30 kg/m² [2]. In obesity, a continuous surplus of calories is stored as body fat, and eventually the excess weight increases risks of developing secondary diseases such as cardiovascular disease and Type II diabetes [2].

Obesity is a global burden; weight loss can improve health. Lasting lifestyle changes have remained a challenge [3] and several approaches have been tested to pharmaceutically treat obesity. But so far, there are no effective treatments that hinder weight gain or promote weight loss without side effects [4]. Current medications either decrease appetite or reduce the gastrointestinal uptake of calories by enzymatic inhibition, thereby reducing the efficiency of the food. An innovative approach would be to find a treatment with a safe way to reduce food efficiency; reducing the uptake and subsequent conversion of calories into excessive adiposity.

Certain foods and dietary supplements are able to affect energy metabolism. In the search for active components silicon dioxide (silica) has been proposed [5]. Silica as a food supplement has been shown to decrease blood cholesterol levels in rats [6,7] and humans [8], an effect that has been linked to increased fecal fat [7] and bile excretions [6]. More specifically, it has previously been indicated that MSPs can cause weight loss in obese mice [9].

MSP is a type of synthetic amorphous silica (SAS) with particularly interesting properties for biomedical applications. MSP distinguishing properties are its generous surface area, increasing the possibilities of interacting with biomolecules, as well as its organized pore structure with pore diameters in the range of 2–50 nm. These properties, combined with high stability and biocompatibility, enable biomedical applications [10]. For example, both drugs [10,11] and proteins [10] have *in vitro* been loaded or immobilized [12] into the pores of MSPs in order to enhance delivery, or control the components release. However, it is not known in what ways MSPs with unfilled pores react in a physiological setting and whether they influence uptake of nutrients *in vivo*. We here hypothesized

that MSP physiochemical characteristics, such as pore architecture, could affect intestinal energy uptake and food efficiency, as suggested by weight loss through MSPs in obese mice [9].

MSPs are chemically the same as already approved food additives (E551 under EU regulations), described in the pharmacopoeia in the form of SAS [13,14]. E551 is commonly applied as anticaking agent in powdered foods such as spices and nondairy coffee creamer and as fining agents in wine, beer and juice. In the pharmaceutical industry, SAS is used as an excipient, binder, anticaking agent, adsorbent, disintegrating or flow agent. Silica has the CAS number: 112926-00-8 and EC list number: 601-214-2. Several reviews and assessments of silicon dioxide safety data have been published [15–17]. Silica is considered safe for oral administration in both mice and men.

We aimed to investigate the metabolic effects of tailored MSPs with precisely controlled pore size in a C57BL/6 thermoneutral diet-induced obesity (DIO) mouse model measuring food efficiency, weight and metabolic profile.

Materials & methods

Synthesis of mesoporous material

MSP SM0016 was synthesized by a modified method as reported previously [18]. All the chemicals used in the synthesis were purchased from Sigma-Aldrich (MO, USA). In brief, a meso-structure templating agent (P123, a triblock copolymer with average MW = 5800, PEO₂₀PPO₇₀PEO₂₀) was dissolved in aqueous hydrochloric acid, with acid concentration equivalent to 2.0 M. Complete dissolution of P123 was followed by addition of tetraethyl orthosilicate (TEOS) under vigorous stirring at 40°C. The final molar ratio of the solution was P123:TEOS:HCl:H₂O : 0.02:1.00:7.05:166.33. Synthesis was kept static at 40°C for 24 h and further hydrothermal treated for 48 h at 100°C. Filtered, washed and dried material was subjected to calcination (550°C in air) to remove the organic template and generate the open porous network.

Following initial studies, MSPs SM0021 and SM0023 were prepared using a modified synthesis. P123 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 the solution was P123:TEOS:HCl:H₂O : 0.02:1.00:6.32:234.62. Synthesis was kept static at 40°C for 20 h and further hydrothermal treated for 20 h (SM0023) or 46 h (SM0021) at 100°C. Filtered, washed and dried material was subjected to calcination (550°C in air) to remove the organic template and generate the open porous network.

Commercial silica purchased from AGC Si-Tech Co., Ltd (Fukuoka, Japan) MS Gel EP-DF grade, with average particle size of 5 µm and pore size of 8 nm according to the manufacturer's description, from here on abbreviated (EPDF 5/80) was used as a comparator to investigate if a commercially available material with comparable pore size characteristics would exert the same effects.

Material characterization

Nitrogen sorption analysis was performed in order to characterize the silica particles in terms of surface area, pore volume and pore size [19]. The analysis was performed at liquid nitrogen temperature (-196°C) using a TriStar II volumetric adsorption analyzer (Micromeritics Instrument Corp., GA, USA). The samples were degassed to empty the pores from air and moisture on a degassing station prior to analysis. Degassing was performed at 150°C under vacuum for a minimum of 15 h, sample weight after the degassing was used for calculating the Brunauer–Emmett–Teller (BET) surface area and pore volume. Data analysis was performed with the Tristar II 3020 software (Micromeritics Instrument Corp., GA, USA). BET surface area was calculated from adsorption isotherm at a relative pressure (p/p°) of <0.2. Total pore volume was recorded at a relative pressure (p/p°) = 0.99. Pore size was obtained by applying the density functional theory (DFT) method to the adsorption isotherm, assuming a cylindrical pore model for SM0016, SM0021 and SM0023, whereas slit-fitting was used for EPDF 5/80 owing to its worm-like pore structure. DFT takes into account known properties of the adsorptive and utilizes an integral equation of adsorption of first degree. For the calculation of pore size distribution, it is assumed that each pore contributes independently toward the total adsorption [20].

Pore structure was characterized by low-angle x-ray diffraction (XRD) on a powder PANalytical X'Pert Pro diffractometer (PANalytical, Karlsruhe, Germany), equipped with Cu K α radiation source, operated at 45 Kv and 40 mA, scanning at low angle from 0° to 8° 2 θ with 0.02° step size under transmission mode, with no background. Prior to analysis, sample insert was prepared by placing one piece of transparent Mylar C foil over the insert and clamped with a plastic ring of smaller diameter. MSP sample was loaded onto the transparent foil, spreading it evenly and a second foil film was used to sandwich the powder between the two layers of foil. The second foil was fixed with another plastic ring. This sample insert was loaded into the holder and fixed with a steel holder ring.

Pore structure was observed by transmission electron microscopy using a JEOL JEM-2100F instrument (JEOL Ltd, Tokyo, Japan) equipped with LaB6 emission gun. Prior to analysis, the sample was dispersed and sonicated in ethanol solution, and a drop of this suspension was transferred to a holey carbon coated copper grid.

Scanning electron microscopy (SEM) using a JEOL JSM-7401F (JEOL Ltd, Tokyo, Japan) equipped with Schottky-type field emission gun was used to characterize the particle agglomerates and morphology.

Particle size measurements were determined using a Malvern[®] Zetasizer Nano ZS (Malvern Instruments Ltd, Worcestershire, UK) by dynamic light scattering (DLS). Prior to particle size measurements, particles were dispersed in phosphate buffer of pH 7.3 and sonicated for 2–4 min. Particle size was calculated as average of triplicated measurement of the z-average hydrodynamic diameter.

Animals

9-week-old male C57Bl/6N mice (Scanbur, Sweden) were single caged and held at 12 h:12 h light/dark cycle at thermo-neutral temperature, 30°C, with 50% humidity. The sample size was based on assessment the statistical variability of previous experiments. Mice were allocated by random assignment into experimental groups with free access to their respective diets and water during the entire experiment. There was no blinding of group allocations. The study was approved by the animal ethics committee of the North Stockholm Region (Dnr N240/14).

Diet-induced obesity

Mice were fed chow diet (R70) or Research Diets D12451 high-fat high-caloric (HFHC) diet in which 45% of the calories come from fat. HFHC diet was administered alone or containing admixed MSPs. Weekly, food intake and bodyweight were measured by scale, calorie intake was calculated by multiplying the food weight with stated energy densities of the diets. Water intake was measured during week 5 and 6. Fecal samples were collected during week 6 and 7 and left to dry at room temperature before weighing. Body fat and lean mass was measured by MRI (EchoMRI-700/100 body composition analyzer; Echo Medical Systems, NY, USA). Food efficiency (% of food intake stored as fat) was calculated as $\Delta fat\ mass\ (kJ)/food\ intake\ (kJ) \times 100$ where the energy density for fat mass is 37 kJ/g.

Mesoporous silica treatment

To manufacture MSP-supplemented diets, the MSP was dried, weighed and mixed in a food processor with pulverized, weighed HFHC pellets and new pellets were prepared in the same format as the original pellets. The 4% (w/w) MSP supplement is an increased dose in comparison with a previous model used for studying weight gain [9].

Glucose tolerance test

Animals were fasted 5 h prior to an intraperitoneal injection of glucose (1 g/kg lean body mass). Blood samples were obtained from the tail at 0, 15, 30, 60, 90 and 120 min for glucose determination using a glucometer (MultiCare-In, Pedithealth, Finland). In animals where blood glucose did not increase by at least 1 mmol/l from 0 to 15 min were excluded from the data analysis.

Termination & blood sampling

At the end of the study, blood was collected via cardiac puncture following cervical dislocation. Blood samples were allowed to clot at room temperature for 1 h before centrifugation at $12,000 \times g$ for 15 min at 4°C. The separated serum was immediately stored at -80°C until further analysis. Bio-Plex Pro[™] mouse diabetes 8-plex assay (Bio-Rad laboratories, Solna, Sweden) was used to analyze insulin, ghrelin and leptin levels in serum. The Bio-Plex Pro mouse cytokine 8-plex assay (Bio-Rad laboratories) was used to analyze IL-1b, IL-5, IL-10 and TNF-α levels in serum.

Results

Physiochemical characteristics of the MSPs

To identify the parameters correlated with metabolic effects, the MSPs were characterized in terms of surface area, pore volume, pore size, morphology, particle size and pore structure, using nitrogen adsorption analysis, SEM, DLS and low-angle XRD. To increase accuracy and precision of MSP synthesis while scaling-up the process, the manufacturing was transferred from an academic lab (particle SM0016) to a small size industrial laboratory

Table 1. Physicochemical properties of synthesized mesoporous silica materials SM0016, SM0021, SM0023 and commercial mesoporous silica EPDF 5/80.

Properties measured	SM0016	SM0021	SM0023	EPDF 5/80
BET surface area (m ² /g)	691	721	684	462
Total pore volume (cm ³ /g)	0.8	1.1	0.9	0.9
Mean pore size (nm)	10.1 [†]	11.2 [†]	9.6 [†]	9.3 [‡]
Particle size (μm)	0.9	1.0	0.9	3.1
Unit cell parameter (nm)	11.2	11.6	11.0	15.0
d ₁₀₀ spacing (nm)	9.7	10.1	9.6	13.1

[†] Density functional theory cylindrical fit.
[‡] Density functional theory slit fit.
 BET: Brunauer–Emmett–Teller.

(particles SM0021 and SM0023). In the enlarged and refined setting, parameters of the manufacturing process were adjusted to precisely tailor the particles.

BET surface area, total pore volume and pore size of EPDF 5/80, SM0016, SM0021 and SM0023 are presented in Table 1. The BET surface area and pore volumes of the synthesized SM0016, SM0021 and SM0023 are in accordance with previous reports of this class of MSPs [18]. The nitrogen adsorption–desorption isotherm and pore size distribution data for all batches are presented in Figure 1A & B. The nitrogen adsorption–desorption isotherm of all the studied materials (Figure 1A) was in accordance with mesoporous silica materials [21] and can be classified as type IV according to the International Union of Pure and Applied Chemistry nomenclature, with clear hysteresis loops. 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. SM0016, SM0021 and SM0023 are clearly type H1 hysteresis loops (Figure 1A), which is associated with well-defined cylindrical pores. On the contrary, EPDF 5/80 hysteresis is more closely related to type H2, which indicates a broader pore size distribution having poor connectivity, order and shape [22].

The mean pore size of the studied particles was in the range 9.3–11.2 nm (Table 1) which has previously been indicated to have metabolic effects [9]. However, the particles differ in their pore size distributions (Figure 1B). As expected from the shape of the hysteresis loops discussed above, EPDF 5/80 has a broad pore size distribution while SM0016, SM0021 and SM0023 have a narrower pore size distribution although SM0016 has an asymmetrical pore size distribution. The pore size distribution of synthesized silica SM0021 and SM0023 was narrow and symmetrical, indicating improved control over pore size.

The low angle XRD pattern in Figure 1C shows peaks that can be indexed on the basis of 2D hexagonal pore geometry for SM0016, SM0021 and SM0023, with SM0023 having higher intensity 110 and 200 diffraction compared with SM0016 and SM0021, indicating a more organized porous structure. The hexagonal pore geometry of SM0023 was also confirmed by transmission electron microscopy observation (Supplementary Figure 1).

The diffraction from commercial EPDF 5/80 shows a single broad peak centered around 0.6° 2θ, with corresponding d₁₀₀ spacing of 13.1 nm, larger than the d₁₀₀ spacing for SM0016, SM0021 and SM0023 at 9.7, 11.6 and 11.0 nm, respectively (Table 1). The unit cell parameters (α) were calculated using (2/√3) d₁₀₀ and are presented in Table 1. Calculated values for SM0016, SM0021 and SM0023 are in accordance with previous reports for this class of MSP [23].

Overall, the N₂ sorption and XRD data in Figure 1A–C is in remarkable agreement for a structurally amorphous pore structure for EPDF 5/80, with broad pore size distribution and poor connectivity, while SM0016, SM0021 and SM0023 have a better porous structural organization.

Particle morphology determined by SEM revealed large agglomerates of several micrometers composed of rod-shaped particles of about 1 μm in length for SM0016, SM0021 and SM0023 whereas commercial EPDF 5/80 is composed of monodisperse spherical particles (Figure 1D). DLS measurements further revealed that the average hydrodynamic sizes in phosphate buffer of particles from synthesized batches SM0016, SM0021 and SM0023 were 0.9–1.0 μm, while EPDF 5/80 average hydrodynamic diameter was 3.1 μm, which are consistent with the observation of the SEM images (Figure 1D).

Figure 1. Physiochemical characterization of mesoporous silica particle materials. (A) Nitrogen adsorption-desorption isotherms. **(B)** Pore size distribution measured via density functional theory. **(C)** Low angle x-ray diffraction patterns with peaks 110 and 200 raised by a factor of 5 for clarity purposes. **(D)** Scanning electron microscopy micrographs of synthesized silicas (SM0016, SM0021 and SM0023) and commercial silica (EPDF 5/80), from left to right: SM0016, SM0021, SM0023 and EPDF 5/80.

Positive weight gain in a DIO model

To verify the DIO model in thermoneutrality, one group of six male C57Bl/6 (henceforth referred to as the HFHC control) was given a HFHC diet for 7 weeks while tracking their metabolic parameters to monitor their development compared with a group on standard chow diet ($n = 6$) (Figure 2A). During the first week, the HFHC control showed a larger caloric intake compared with the chow group, 395 ± 15 kJ compared with 261 ± 13 kJ, a caloric intake difference that persisted throughout the diet intervention although in a smaller scale.

Weight was monitored weekly throughout the study and, as expected, all mice grew and gained weight during the treatment period. The mean weight increase over the 7-week treatment was 6.05 ± 0.42 g for the chow group versus 16.12 ± 0.70 g for the HFHC control (Figure 2B). Similar differences were observed for body fat increases, which were 4.05 ± 0.50 g for the chow and 13.81 ± 0.67 g for control. Lean mass changes were insignificant in all groups throughout the experiment, showing that mice will gain predominantly fat weight in form of adipose tissue with HFHC feeding (Figure 2C & D). These results validate the weight gain in the obesity model.

Figure 2. Weight gain and glucose tolerance in a diet-induced obesity model with silica treatment and compared with chow diet. (A) Outline of study where male C57BL/6 mice were fed control HFHC ($n = 6$), HFHC containing 4% SM0016 ($n = 7$) or chow diet ($n = 6$), for 8 weeks. Animals were weighed weekly and MRI was used to determine body fat and lean masses. (B) Bodyweight development throughout the study. (C) Absolute body fat increases after 7 weeks of treatment. (D) Body fat weight development throughout the study. (E) GTT before treatment start where control ($n = 5$), SM0016 ($n = 7$) and chow ($n = 6$) react similarly to a glucose load. (F) GTT after 7 weeks of treatment: control ($n = 4$), SM0016 ($n = 6$) and chow ($n = 6$). Data are represented as mean $\pm$ standard error. The $p < 0.05$ with a two-tailed t-test or two-way analysis of variance was considered significant, data had comparable variance and met the assumptions of the tests.

* $p < 0.05$.

GTT: Glucose tolerance test; HFHC: High-fat high-caloric; ns: Not significant.

Confirmed animal wellbeing with MSP intake

The 7-week DIO model at thermoneutrality was applied to investigate effects of the MSP SM0016 on obesity development. Therefore, in addition to the chow and HFHC control groups, one group ($n = 7$) received a 4% SM0016 supplement in the HFHC diet (HFHC diet with SM0016).

The overall well-being of the animals was confirmed by an expected weight gain profile and no start-of-treatment weight loss due to feed aversion was observed. This is in contrast to what has been observed at initiation of treatment with obesity drug Orlistat [24].

To detect if the digestibility of the diet containing MSP was altered, the weekly feces excretion was collected and weighed. As diet intake was recorded, the weekly MSP intake could be calculated. Uptake through the gastrointestinal epithelium is inversely correlated to particle size [25] and ingested particles of sizes above 1000 nm are unlikely to be taken up [26]. Instead they pass through the gastrointestinal system and out in the feces. The weight of MSP could then be deducted from the total feces weight, resulting in a theoretical MSP free feces weight. The MSP free feces weights showed no significant differences between groups (data not shown). Additionally, thermogravimetric analysis of the feces showed that the difference in inorganic material between HFHC control and MSP-supplemented groups roughly corresponded to the ingested MSP dose, confirming that MSP of this size pass through the gastrointestinal system without absorption. An objective difference in stool width was noted, but further studies would be needed to confirm this observation. Addition of SM0016 in the diet did not affect water consumption, which corresponded to 2.23 ± 0.08 ml/day for the HFHC control group and 2.18 ± 0.07 ml/day for HFHC 4% SM0016 group, arguing that the MSP addition does not affect the animals' drinking behavior.

Further confirming silica's safety, blood samples were taken at sacrifice to control for signs of inflammation. Serum cytokines showed no difference between the HFHC 4% SM0016 and HFHC control (Supplementary Figure 2), indicating that the weight differences are not due to any immunological reaction. Silica is recognized as safe in use as a food supplement for human consumption and the results presented here confirm the safety profile.

MSPs SM0016 attenuate obesity development

Weight and adiposity development were followed weekly. The group receiving 4% SM0016 supplementation in the HFHC diet had a significantly lower weight gain and suppressed adipose tissue formation compared with HFHC control. Mean weight increase was 13.40 ± 0.96 g for the SM0016 group, which is a 17% lower increase than the HFHC control group. Fat mass increases were 11.15 ± 0.84 g for SM0016 compared with 13.81 ± 0.67 g for HFHC control, while lean mass remained constant (Figure 2C & D). The combined data show that the reduced weight gain in the SM0016 group compared with the HFHC control group was due to attenuated body fat accumulation.

MSPs SM0016 ameliorate the metabolic profile

The glucose homeostasis is typically impaired in C57BL/6 mice under HFHC feeding conditions [27], an effect that can be evaluated through a glucose tolerance tests. At the start of the study, the groups showed similar acute responses to glucose in a glucose tolerance test (Figure 2E). Whereas after 7 weeks with obesogenic diet, the HFHC control group showed higher glucose levels 60 min after glucose load than SM0016 supplemented ($p = 0.0162$). Surprisingly the HFHC 4% SM0016 group showed a glucose tolerance curve similar to the chow group (Figure 2F), indicating that mice on HFHC 4% SM0016 retain their sensitivity to glucose and insulin.

To compare hormonal profiles and see if the differences in adiposity between the SM0016 group and the HFHC control group were accompanied by changes in hormone levels, nonfasted blood samples were collected at the end of the study. Ghrelin is produced and secreted by the stomach upon fasting, stimulating food intake [28], plasma levels have been shown to negatively correlate with obesity [29]. The HFHC 4% SM0016 group tended to show higher ghrelin levels (5.40 ± 0.61 ng/ml) than the HFHC control group (3.85 ± 0.52 ng/ml) although the difference was not significant. Leptin is a hormone secreted by the adipose tissue, signaling satiety and inhibiting the food intake pathways ghrelin stimulates [30]. Its circulating plasma levels directly correlate to adiposity [31] and prolonged high fat diet exposure will decrease the body's responsiveness to leptin [32]. HFHC 4% SM0016-treated animals showed significantly lower leptin levels (29.62 ± 2.03 ng/ml) than HFHC control (54.93 ± 6.46 ng/ml). The results show that the MSP reduced adiposity was linked to an ameliorated metabolic profile.

Figure 3. Energy intake and food efficiency with high fat high caloric diet and silica treatment. Male C57BL/6 mice were fed HFHC (n = 6), HFHC containing 4% SM0016 (n = 7) for 7 weeks. **(A)** Cumulative diet intake during 7 weeks of treatment. **(B)** Cumulative food intake, represented in the food's energy content, during 7 weeks of treatment, calculated as HFHC intake in gram multiplied by energy density as per manufacturers specification. **(C)** Food efficiency over 7 weeks of treatment calculated as fat mass increase in gram * 37 kJ/g divided by kJ energy intake, multiplied by 100 for a percentage. Data are represented as mean $\pm$ standard error. The $p > 0.05$ with a two-tailed t-test was considered significant, data had comparable variance and met the assumptions of the tests.

* $p < 0.05$; *** $p < 0.001$.

HFHC: High-fat high-caloric; ns: Not significant.

Reduced food efficiency with MSPs SM0016

Diets were given *ad libitum* and the resulting feed intake was measured weekly. There was a significant difference in diet intake between HFHC control and HFHC 4% SM0016 (Figure 3A), but when accounting for the MSP in the diet we saw no difference in food energy intake. The cumulative food energy intake for the HFHC control group during 7 weeks of treatment was 2241 ± 75 and 2384 ± 68 kJ for the HFHC 4% SM0016 group represented as mean $\pm$ standard error of the mean (Figure 3B). The similarities in intake suggest that the supplementation with SM0016 neither affects satiety and appetite nor the palatability of the diet but exerts its effect through the gastrointestinal uptake of energy.

Additionally, we determined food efficiency, calculated as fat mass increase in kJ (37 kJ/g fat tissue) per kJ energy ingested [33]. The food efficiency was significantly reduced in the HFHC 4% SM0016-supplemented group compared with HFHC control (Figure 3C). The food efficiency rate during the first 7 weeks of treatment was $22.79 \pm 0.73\%$ for the HFHC control group and $17.21 \pm 0.95\%$ for the HFHC 4% SM0016 group. This further implies that MSP supplementation affects energy uptake in the gastrointestinal tract.

Specificity of the MSP effect on bodyweight

To assess the impact of specific particle characteristics, the now verified obesity model was applied to evaluate the metabolic effects of additional MSPs. At the age of 9 weeks, 35 male C57Bl/6 mice were randomized into five groups: a control group receiving HFHC diet and four groups with different silica treatments called HFHC 2% SM0023, HFHC 4% SM0023, HFHC 4% SM0021 and HFHC 4% EPDF 5/80. Treatment groups received HFHC diet with 4% (w/w) of the respective MSP except the group 2% SM0023 getting HFHC diet with a mixture consisting of 2% (w/w) SM0023 and 2% (w/w) SM0021 (Figure 4A).

Bodyweight and fat mass were measured weekly and interestingly, mice receiving 4% EPDF 5/80 and 4% SM0021 supplemented in HFHC diet showed no difference in total weight or adipose tissue formation compared with the HFHC control, gaining 9.4 ± 1.0 , 9.2 ± 0.6 and 9.4 ± 1.2 g, respectively (Figure 4B). The 4% EPDF 5/80 and 4% SM0021-treated groups behaved similarly to the control group in all subsequent parameters measured.

Figure 4. Weight gain and food efficiency in diet-induced obesity with improved silica treatment. (A) Outline of study where male C57BL/6 mice were fed control HFHC ($n = 7$), HFHC containing 2% SM0023 ($n = 7$), HFHC containing 4% SM0023 ($n = 7$), HFHC containing 4% SM0021 ($n = 7$) and HFHC containing 4% EPDF 5/80 ($n = 7$) for 8 weeks. Animals were weighed weekly and MRI was used to determine body fat and lean masses. (B) Fat mass increase after 7 weeks of treatment as percentage of HFHC control. Significant difference between HFHC 4% SM0023 and HFHC control using one-way analysis of variance with Dunnett's multiple comparison test. (C) Bodyweight development throughout the study. (D) Absolute body fat increases after 7 weeks of treatment. (E) Body fat weight development throughout the study. (F) Cumulative food intake in kJ during 7 weeks of treatment, calculated as food intake in gram multiplied by energy density per manufacturer's specification. (G) Food efficiency over 7 weeks of treatment calculated as fat mass increase in gram $\times$ 37 kJ/g divided by kJ energy intake, multiplied by 100 for a percentage. Data are represented as mean $\pm$ standard error. The $p > 0.05$ with a two-tailed t-test was considered significant, data had comparable variance and met the assumptions of the tests.

* $p < 0.05$.

GTT: Glucose tolerance test; HFHC: High-fat high-caloric; ns: Not significant.

Whereas weight development of the HFHC 4% SM0023-supplemented group (Figure 4C & D) resulted in a significantly lower adipose tissue formation 6.5 ± 0.5 g (Figure 4B & E) compared with control group. In addition, the group receiving 2% SM0023 displayed a weight gain of 7.9 ± 0.9 g; intermediary of the higher 4% SM0023 dose and HFHC control, indicating a dose dependency of the SM0023 particles (Figure 4E). These results suggest that the MSP effect on bodyweight and adipose tissue is particle-specific.

Positive impact of MSPs SM0023 on metabolic profile

Having shown that the HFHC 4% SM0023-treated group showed a significant lowering of adipose tissue formation, additional metabolic parameters were measured. Insulin regulates glucose uptake into tissues as blood glucose rises, in a prediabetic state, the sensitivity to insulin slowly decreases, leading to higher levels of circulating hormone. Here the 4% SM0023 group had lower levels, 6.36 ± 0.49 ng/ml, of circulating insulin compared with the control group 9.51 ± 1.87 ng/ml. Although not statistically significant, this could indicate that the 4% SM0023-treated mice have a higher sensitivity to insulin, in other words, improved glycemic control. However, in contrast to the data from SM0016 presented above, the groups showed no significant differences in a glucose tolerance test (Supplementary Figure 3). Similar to SM0016 treatment, ghrelin levels are reduced by HFHC 4% SM0023 from HFHC control at 27.63 ± 5.22 to 36.39 ± 5.33 ng/ml. Also, for leptin, the pattern from SM0016 was repeated as HFHC 4% SM0023 was 16.91 ± 1.88 ng/ml, which is significantly lower than HFHC control plasma leptin levels 32.76 ± 7.44 ng/ml. This establishes that MSP with the right physiochemical characteristics reduce obesity and improves the hormonal profile.

Tailored MSPs SM0023 reduce food efficiency

Assessing caloric intakes, the cumulative food intake was recorded over 7 weeks and did not differ between groups (Figure 4F) indicating that MSP do not affect appetite. Food efficiency was calculated to evaluate whether an altered balance between food intake and adipose tissue growth [26] could be seen. The control HFHC group showed a food efficiency of $16.07 \pm 1.90\%$ whereas HFHC 4% SM0023 resulted in a significantly lowered food efficiency of $10.81 \pm 0.70\%$ of energy eaten stored as fat (Figure 4G). In addition, a dose dependent effect was detected as with the 2% SM0023 treatment the food efficiency resulted in $12.36 \pm 1.31\%$. The other MSP tested; SM0021 and EPDF 5/80, showed no effect on food efficiency, again pointing toward the importance of the right physiochemical characteristics of functional MSPs to reduce food efficiency.

Discussion

To investigate the metabolic effects of different MSPs with similar pore size, we used a DIO model in thermoneutrality. The obesity model builds on West *et al.* showing that in male C57Bl/6, obesity is developed within 7 weeks of diet intervention [34] and Surwit *et al.* emphasized the strain's predisposition to impaired glucose metabolism with long-term HFHC feeding [27]. Additionally, housing mice in thermoneutrality at 30°C removes the confounding factor of using energy for heat regulation and further increases the positive energy balance [35], thereby increasing the ability to detect effects of treatment, important when investigating putative changes in gastrointestinal energy uptake. All groups with HFHC-based diets increased their caloric intakes, confirming the C57Bl/6 strain predisposition to hyperphagia at the start of HFHC diet [34]. The resulting weight gain confirmed the amplitude, accuracy and validity of the obesity model [27].

Alongside verifying obesity development, the 7-week diet intervention model was applied to observe if adiposity could be attenuated and metabolic parameters improved with a range of MSP supplementations. A metabolic effect in the 7-week setting with *ad libitum* HFHC diet would be of large clinical relevance, as this mimics overweight in humans, largely dependent on an excessive caloric intake [36].

Herein, the MSP-supplemented groups received a 4% addition, leading to an approximate daily intake of 0.1 g silica, an intake that corresponds to 2.5–4 g/kg bodyweight as the mouse weight increases from approximately 25 to 40 g during the 7-week period. Our results showed unaffected lean weight, water intake and blood cytokines which indicate the safety of the MSP treatments. This is corroborated by previous health assessments reporting no toxicity after 2-year oral application in the diet, where no adverse effects were demonstrable for SAS forms (NOAEL[chronic, oral] = approximately 2.5 g/kg bw/day). Following feeding of 0.6–10% silica in the diet for 14 days, no substance-related clinical and histopathological findings at any dose level were observed in rats or mice [15]. The safety of MSP treatment is also substantiated by the fact that the European Food Safety Agency has recently re-evaluated the safety of food grade silica and concluded that there was no indication for toxicity of E551,

although it recognized concerns about nanosize-related toxicity [17]. However, it is worth noting that contrary to some E551 forms, the particles used in this study are micron-sized and do not contain nanoparticles.

In the literature, bioavailability of orally ingested silicon, a major constituent of silica, depends on the solubility of the compound. If solubilized in the gastrointestinal tract, it will be excreted via the kidneys. Silicon content of foods consumed, such as grains and rice, was significantly correlated with urinary silicon excretion [37,38]. Species of silicon and silica that are not readily broken down in the gastrointestinal tract will not be significantly absorbed but instead be excreted in feces [38]. Our feces examinations indicate no systemic uptake of the orally ingested MSP which would improve the safety profile of the supplementations.

Impaired glucose tolerance is linked to obesity, with the reported difference in adiposity between HFHC control and chow groups, altered glucose handling is expected. However, the improved glucose response of SM0016-treated mice cannot be fully explained by a difference in adiposity, this raises the question of whether there is a positive effect of MSP on blood glucose handling. This would imply that MSP reduce adipose tissue formation through decreased energy uptake and, there could be an additional mechanism; such as postprandial blood glucose reduction.

The MSP SM0023 showed no significant changes in glucose responsiveness. It has been reported that HFHC diet will cause impaired glucose metabolism compared with other diets, even when restricting dietary access in a setting such as pair feeding with a low fat diet group, albeit somewhat delayed [39]. Therefore the lack of effect on glucose responsiveness observed with SM0023 could be a timing issue and a robust glucose tolerance alteration could manifest later. However, understanding the differences between the response using different MSP requires further investigation.

Current pharmacological obesity treatment with glucagon like peptide-1 analog liraglutide reduces energy intake by increasing satiety [40]. In contrast, our results show that MSP supplementation does not affect food intake and thus most likely does not influence satiety signaling. Instead, the functional MSP supplements; SM0016 and SM0023, are similar to treatment with the lipase inhibitor orlistat, which does not affect appetite. Orlistat decreases weight through a reduced gastrointestinal lipid availability and absorption [41]. Orlistat shows effects on weight loss and preventing weight regain, but the reduced lipase activity is associated with side effects such as oily stools and fecal urgencies [42], side effects not seen with silica supplementation. Additionally, functional MSP supplementation might mimic the intake of dietary fiber or resistant starch, leading to increased fecal energy excretion [43,44].

The notion that materials can reduce gastrointestinal availability and subsequently weight gain [45] makes the assessment of the food efficiency interesting. Our results demonstrate that the effect on food efficiency is specific for the materials SM0016 and SM0023. SM0016 reduced food efficiency by approximately 25% and SM0023 reduced food efficiency with 33%, indicating an improved reduction with adjustments of the material synthesis. This clearly indicates that the mechanism by which functional MSPs reduce obesity development is connected to gastrointestinal energy uptake.

It has been hypothesized that MSP with specific physiochemical properties affect weight through modulation of lipase activity [9] which would reduce fat uptake and limit the formation of adipose tissue. We noted that treated mice showed no sign of adverse reactions such as steatorrhea, indicating that the mechanism would be milder than full lipase inhibition. We speculate that the MSP could inhibit several digestive enzymes to some extent, leading to reduced digestion. The pore size and pore volume of the MSP could be critical parameters. However, MSP effects on enzymes and the dependence on specific silica properties have so far only been investigated *in vitro* with results pointing in different directions [46,47]. Further *in vitro* and *in vivo* experiments are needed to elucidate the involvement of enzyme inhibition.

Based on our previous work, we hypothesize that all MSP with pore sizes of approximately 10 nm [48] would have effects in a DIO mouse model. However, this was not the case since only two out of four particles tested-induced effect. Both EPDF 5/80, which consists of spherical monodispersed particles showing a broad range of pore sizes peaking at 9.3 nm and rod shaped SM0021 with a slightly larger pore size and narrower pore size distribution than EPDF 5/80, did not cause any reduction in obesity. We hypothesize that well defined structure of pores, tight pore size distribution and pores connectivity are important factor in achieving a specific physiological effect. Commercial EPDF 5/80 does not fit those criteria; its pore arrangement is amorphous, it has a broad pore size distribution and poor connectivity. Interestingly, SM0021 physiochemical properties are close to those of MSP SM0016 and SM0023, this indicates that small changes in physiochemical parameters have large effects on the obesity reduction. We speculate that SM0021 pore structure has some level of constraints, causing it to have no physiological effect, although different characterization methods might be needed to highlight the differences between SM0021 and the effective MSP SM0016 and SM0023.

Interestingly, rod-shaped, functional, MSP SM0016 and SM0023 both showed significant positive effects. SM0016 was produced in a small-scale lab and SM0023 was the result of enlarged and adjusted synthesis with narrow pore size distribution, with peaks at 10.1 and 9.6 nm respectively. This suggests that pore size distribution and pore structure are critical parameters for biological effects. However, we cannot exclude that other parameters are involved, and further characterization will be needed to conclude on the critical parameters.

The combined metabolic data clearly show that MSPs with highly specific characteristics induce obesity reduction and metabolic effects. It shows that the reduced adiposity is dependent on small changes on specific physiochemical characteristics of the functional particles. *In vitro* studies have shown that there are differences between distinct mesoporous materials in pore loading ability and enzymatic interactions [49]. To understand which parameters are paramount for the obesity reduction, we will perform further *in vitro* and *in vivo* studies with MSP having different characteristics. Understanding the mechanism of action leading to the weight gain reduction and metabolic effects would be of high importance due to the impact functional MSP could have on the treatment of obesity.

Conclusion

The main findings presented here are that food efficiency could be reduced by 33% and weight gain significantly attenuated with tailored MSPs in the diet. Furthermore, we ascertained the specificity of the effect, as it is not seen in commercial MSP or differently produced MSP.

The presented mouse studies have shown promising effects on metabolic parameters. Importantly, a reduction of food efficiency to this level has the potential to reduce human obesity and related risk factors.

Future perspective

Obesity is now seen as a global pandemic, and new safe and effective treatments are highly needed. The data presented in this study suggest that tailored MSPs could be used to treat obesity in humans, especially when taking into account the excellent established safety profile of silica particles. Furthermore, new *in vitro* and *in vivo* studies will help decipher the mechanisms involved, and establish which physicochemical properties, in addition to pore size, are critical for the metabolic effects. At last, clinical trials are underway, investigating safety and efficacy in humans, underscoring the importance of this novel approach for the treatment and prevention of obesity.

Summary points

- Oral intake of mesoporous silica particles attenuates adipose tissue accumulation in a diet-induced obesity model.
- The mechanism of adipose tissue attenuation is through lowering of food efficiency.
- Attenuation of adipose tissue accumulation is dose dependent.
- Oral intake of mesoporous silica particles improves the metabolic profile.
- Metabolic effect is specific to precisely engineered mesoporous silica particles.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/nnm-2019-0262

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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