Targeted Microbiome Intervention by Microencapsulated Delayed-Release Niacin Beneficially Affects Insulin Sensitivity in Humans

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Daniela Fangmann,[1] Eva-Maria Theismann,[2] Kathrin Türk,[1] Dominik M. Schulte,[1] Isabelle Relling,[1] Katharina Hartmann,[1] Julia K. Keppler,[2] Jörg-Rainer Knipp,[2] Ateequr Rehman,[3] Femke-Anouska Heinsen,[3] Andre Franke,[3] Lennart Lenk,[4] Sandra Freitag-Wolf,[5] Esther Appel,[6] Stanislav Gorb,[6] Charles Brenner,[7] Dirk Seegert,[8] Georg H. Waetzig,[8] Philip Rosenstiel,[3] Stefan Schreiber,[1,3] Karin Schwarz,[2] and Matthias Laudes[1]

OBJECTIVE

Gut microbiota represent a potential novel target for future prediabetes and type 2 diabetes therapies. In that respect, niacin has been shown to beneficially affect the host-microbiome interaction in rodent models.

RESEARCH DESIGN AND METHODS

We characterized more than 500 human subjects with different metabolic phenotypes regarding their niacin (nicotinic acid [NA] and nicotinamide [NAM]) status and their gut microbiome. In addition, NA and NAM delayed-release microcapsules were engineered and examined in vitro and in vivo in two human intervention studies (bioavailability study and proof-of-concept/safety study).

RESULTS

We found a reduced α-diversity and *Bacteroidetes* abundance in the microbiome of obese human subjects associated with a low dietary niacin intake. We therefore developed delayed-release microcapsules targeting the ileocolonic region to deliver increasing amounts of NA and NAM to the microbiome while preventing systemic resorption to avoid negative side effects (e.g., facial flushing). In vitro studies on these delayed-release microcapsules revealed stable conditions at pH 1.4, 4.5, and 6.8, followed by release of the compounds at pH 7.4, simulating the ileocolonic region. In humans in vivo, gut-targeted delayed-release NA but not NAM produced a significant increase in the abundance of *Bacteroidetes*. In the absence of systemic side effects, these favorable microbiome changes induced by microencapsulated delayed-release NA were associated with an improvement of biomarkers for systemic insulin sensitivity and metabolic inflammation.

CONCLUSION

Targeted microbiome intervention by delayed-release NA might represent a future therapeutic option for prediabetes and type 2 diabetes.

Rather than being viewed as simple commensals, the gut microbiome is now seen as playing an active role in the control of energy homeostasis and in the mediation of the adverse consequences of obesity (1). Several studies in the recent past in humans and rodents revealed that obesity is associated with a reduction in *Bacteroidetes* (2–4) and with a lower diversity compared with healthy and lean subjects (4,5). Of physiological relevance, the composition of the gut microbiota can be altered by diet, because

[1]*Department of Internal Medicine 1, University of Kiel, Kiel, Germany*
[2]*Department of Food Technology, University of Kiel, Kiel, Germany*
[3]*Institute of Clinical Molecular Biology, University of Kiel, Kiel, Germany*
[4]*Institute for Experimental Cancer Research, University of Kiel, Kiel, Germany*
[5]*Institute of Medical Informatics and Statistics, University of Kiel, Kiel, Germany*
[6]*Zoological Institute, University of Kiel, Kiel, Germany*
[7]*Department of Biochemistry, University of Iowa, Iowa City, IA*
[8]*CONARIS Research Institute AG, Kiel, Germany*

Corresponding authors: Matthias Laudes, matthias.laudes@uksh.de, Karin Schwarz, kschwarz-2@foodtech.uni-kiel.de, and Stefan Schreiber, s.schreiber@mucosa.de.

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weight loss interventions have been reported to influence the abundance of *Bacteroidetes* (3) and the overall microbial diversity (5,6).

Administration of niacin (nicotinic acid [NA] and nicotinamide [NAM]) has been shown to beneficially effect the host-microbiome interaction in a mouse model (7). Nicotinamide adenine dinucleotide (NAD+) is the central cofactor of metabolism, mediating fuel oxidation, ATP generation, reactive oxygen species (ROS) detoxification, biosynthetic processes, DNA repair, and nutritionally sensitive gene regulation (8). In vertebrates, NAD+ is synthesized de novo from tryptophan and from three vitamin precursors. NAM and NA are the classic NAD+ precursor vitamins. Nicotinamide riboside (NR) was discovered to be a NAD+ precursor vitamin much more recently (9). The NAD+ metabolome has been shown to be dysregulated in obesity and type 2 diabetes in mice (10). Moreover, NR repletion has been shown to blunt weight gain on a high-fat diet (11) and to oppose fatty liver on a high-fat high-sucrose diet (12), largely by increasing the activity of SIRT1, an NAD+-dependent protein lysine deacetylase.

These data on the beneficial effects of niacin on both the gut microbiome and systemic glucose metabolism suggest this micronutrient is an interesting candidate for future targeted microbiome interventions (e.g., to prevent manifestation of type 2 diabetes from prediabetes). However, because the upper gastrointestinal tract efficiently resorbs soluble micronutrients, simply increasing the NA and/or NAM nutritional load would not be expected to deliver these molecules into the ileocolonic region, where most of the microbiome is located (13). The aim of the current study was therefore *1*) to examine NA and NAM in humans in relation to obesity and the gut phylogenome in a large human cohort of >500 well-characterized individuals and *2*) to use a microencapsulation procedure to develop a novel delayed-release system to deliver significant amounts of NA and NAM into the human colon to beneficially affect the gut microbiome and systemic metabolism while preventing systemic side effects.

RESEARCH DESIGN AND METHODS

Study Cohorts and Study Designs

The present investigation included 511 subjects of the Food Chain Plus (FoCus) cohort, which has been previously reported (14). Fasted serum samples and stool samples were collected, and anthropometric measurements were performed within subjects of the FoCus cohort. Further, 481 subjects completed a 12-month retrospective food frequency questionnaire used by the European Prospective Investigation into Cancer Nutrition (EPIC) study was completed by n = 481 subjects (15) to determine niacin (NA+NAM) nutritional intake. Baseline characteristics of the FoCus subset are reported in Table 1.

Two human intervention studies were performed to evaluate NA/NAM microcapsules in vivo: *1*) a bioavailability study including 20 healthy subjects (mean age 26.85 ± 4.86, 50% female, median BMI 22.78 kg/m^2 [interquartile range 21.25; 25.57]) (Supplementary Table 1) and *2*) a proof-of-concept and safety study including 10 metabolically healthy subjects without manifest metabolic diseases and with normal glucose and triglycerides levels (mean age 44.80 ± 11.06, 80% female, BMI 26.67 ± 3.68 kg/m^2) (Supplementary Table 2). In those human interventions, NA and NAM effects observed under different dosages of microencapsulated NA (NA group) or NAM (NAM group) were compared with effects after ingestion of a reference dose of free NA (30 mg) or NAM (900 mg). During the bioavailability study, one subject of the NA group dropped out after 2 study days due to persistent difficulties in blood sample collection. Within the proof-of-concept and safety study, one subject of the NA group was excluded after week 5 because of elevated aspartate transaminase levels, and one subject of NAM group dropped out in week 2 due to an accident not associated with the study.

Subjects were recruited at the University Hospital Schleswig-Holstein (Kiel, Germany). The local ethics committee (Kiel, Germany) approved the niacin interventions (D439/15) and the FoCus study (A108/08), and written confirmed consent was obtained from each subject.

Biochemical Analysis

Blood samples underwent routine laboratory analyses at the central laboratory of the University Hospital Schleswig-Holstein (Kiel, Germany). The HOMA index was calculated as glucose (mg/dL) × insulin (mIU/L)/405. Serum was stored immediately at −80°C. NA and NAM serum levels were measured by liquid chromatography and tandem mass spectrometry (Agilent 1100 HPLC/CTC-PAL Autosampler/Sciex API 4000 Triple Quadrupole) in an external specialized laboratory (Medizinisches Labor Bremen). Systemic metabolic parameters in the serum samples were measured by ELISA using the myostatin test kit (SEB653Hu, Cloud-Clone Corp.), fetuin-A test kit (SEA178Hu, Cloud-Clone Corp.), and osteopontin test kit (SEA899Hu, Cloud-Clone Corp.), following the manufacturer's instructions. Gut microbiome analysis was performed by 16S rDNA amplicon sequencing as described by Heinsen et al. (6).

Production of NA and NAM Microcapsules and In Vitro Evaluation

Microcapsule cores, including NA or NAM (SternVitamin, Ahrensburg, Germany), were prepared. To achieve higher daily doses of NAM, NAM cores were prepared by a ProCell fluidized bed granulator with Vario 3 from an external company (Glatt Ingenieurtechnik, Weimar, Germany). In contrast, NA was applied to Cellets350 in a Mini Glatt fluidized bed coater with bottom spray. In the next step, NA/NAM cores were both coated with an inner shellac (SSB Aquagold, Bremen, Germany) coating (2% weight gain [w.g.]/50% w.g.), an intermediate layer of a pH-modulating substance, sodium bicarbonate for NA (1% w.g.), and citric acid for NAM (1% w.g.), and finally, an outer shellac coating (20% w.g./10% w.g.). Microcapsules were then dried at 50°C in an oven for 1 h. For in vivo studies, NA and NAM microcapsules and NA and NAM powder (free niacin) were filled into standard size 0 gelatin capsules by using a manual capsule filler. Differential dosing was achieved by administration of different amounts of full or partially filled capsules.

Dissolution tests were performed in triplicate with 0.5 g NA or NAM microcapsules in 250 mL simulated gastric fluid (pH 1.4), citrate buffer (pH 4.5), and phosphate buffers (pH 6.8 and 7.4) using a standard dissolution paddle apparatus at 100 rpm and 37°C (DT 70; Pharmatest Group, Hainburg, Germany). Exposure to the release media was set to 1 h at pH 1.4, 0.5 h at pH 4.5, 2 h at pH 6.8, and 1.5 h at pH 7.4. Niacin release was recorded every 30 min by a ultraviolet-visible spectrophotometer at 262 nm (Helios Gamma; Thermo Fisher Scientific, Waltham, MA).

Table 1—Characteristics of the FoCus subset study cohort

	BMI <20 kg/m^2 (n = 66)	BMI 20–25 kg/m^2 (n = 149)	BMI >30 kg/m^2								
			Without T2D (n = 148)	With T2D (n = 148)	P_{total}	P_1	P_2	P_3	P_4	P_5	P_6
Age (years)	45.53 ± 15.58	52.90 ± 10.82	52.82 ± 10.86	52.93 ± 10.83	<0.001	<0.001	<0.001	<0.001	NS	NS	NS
Female sex (%)	86.4	67.1	66.9	66.2	<0.05	<0.01	<0.01	<0.01	NS	NS	NS
Height (m)	1.70 (1.64; 1.75)	1.72 (1.68; 1.79)	1.70 (1.64; 1.78)	1.70 (1.64; 1.80)	NS						
Weight (kg)	55.45 (50.38; 58.43)	66.80 (61.35; 74.40)	111.30 (95.73; 132.20)	123.25 (102.50; 148.08)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
BMI (kg/m^2)	19.09 (18.18; 19.73)	22.81 (21.45; 23.98)	37.08 (32.40; 45.13)	42.80 (36.75; 47.94)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Blood pressure (mmHg)											
Systolic	120.00 (110.00; 130.00)	120.00 (115.00; 130.00)	130.00 (130.00; 140.00)	140.00 (130.00; 140.00)	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	NS
Diastolic	80.00 (70.00; 80.00)	80.00 (70.00; 80.00)	80.00 (80.00; 90.00)	80.00 (80.00; 90.00)	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001
Glucose (mg/dL)	88.00 (85.00; 95.25)	93.00 (87.00; 99.00)	100.00 (91.00; 108.00)	123.00 (104.25; 162.00)	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001
Insulin (mIU/L)	5.25 (3.90; 8.30)	6.50 (5.10; 9.28)	17.20 (11.00; 24.23)	25.50 (15.53; 43.88)	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001
HOMA-IR index	1.14 (0.83; 1.89)	1.49 (1.08; 2.26)	4.15 (2.57; 5.71)	7.87 (4.18; 16.94)	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001
Triglycerides (mg/dL)	68.00 (54.75; 92.50)	87.00 (64.00; 112.50)	121.50 (91.25; 179.25)	166.00 (122.00; 237.75)	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Lp(a) (mg/L)	117.00 (95.00; 361.00)	121.00 (95.00; 320.00)	148.00 (95.00; 378.50)	108.00 (95.00; 324.25)	NS						
IL-6 (pg/mL)	2.15 (1.50; 3.73)	2.50 (1.50; 4.20)	4.00 (2.90; 5.35)	5.20 (3.33; 7.08)	<0.001		<0.001	<0.001	<0.001	<0.001	0.001
CRP (mg/L)	0.90 (0.90; 1.13)	0.90 (0.90; 1.90)	4.35 (1.75; 8.65)	5.85 (2.90; 10.88)	<0.001	NS	<0.001	<0.001	<0.001	<0.001	NS

Data are presented as mean ± SD, as median (interquartile range), or as otherwise indicated. P_{total}, P value for overall comparison. $P_{1–6}$ = pairwise comparison: P_1, BMI <20 kg/m^2 vs. BMI 20–25 kg/m^2; P_2, BMI < 20 kg/m^2 vs. BMI >30 kg/m^2 without T2D; P_3, BMI <20 kg/m^2 vs. BMI >30 kg/m^2 with T2D; P_4, BMI 20–25 kg/m^2 vs. BMI >30 kg/m^2 without T2D; P_5= BMI 20–25 kg/m^2 vs. BMI > 30 kg/m^2 with T2D; P_6, BMI >30 kg/m^2 without T2D vs. BMI > 30 kg/m^2 with T2D. CRP, C-reactive protein; IR, insulin resistance; Lp(a), lipoprotein(a); IL-6, interleukin-6; T2D, type 2 diabetes.

Scanning Electron Microscopy of Microcapsules

Microcapsules from stool samples were isolated, washed in demineralized water, and dried overnight. Afterward, the isolated digested microcapsules and undigested control microcapsules were prepared on a holder with Leit-C conductive carbon cement. Before examination in a Hitachi S-4800 (Hitachi High-Tech, Tokyo, Japan) scanning electron microscope (SEM) at an accelerating voltage of 3 kV, microcapsules were sputter coated with a layer of 8–10 nm gold-palladium using a Leica EM SCD 500 (Leica Microsystems GmbH, Wetzlar, Germany) high-vacuum sputter coater. SEM photographs of the capsules isolated from stool samples were compared with SEM photographs of undigested microcapsules.

Statistical Analyses

Statistical analyses were performed with SPSS 22.0 for Windows software (IBM, Armonk, NY), and graphic data analysis was performed using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA). Data were checked for normality by using Shapiro-Wilk tests and are presented as means ± SDs (normal distribution) or median and interquartile range (not normal distribution). Independent samples *t* tests and Mann-Whitney *U* tests were used to determine differences between groups for continuous variables, and the χ^2 test was used for categorical variables. The Kruskal-Wallis test was used to compare nonparametric data for more than two groups. Welch ANOVA with the Games-Howell post hoc test was used for parametric data with heterogeneity of variances. Spearman correlation analysis was performed according to distribution. Areas under the curve (AUCs) were calculated with GraphPad Prism. If serum levels after 12 h did not match baseline serum levels, the NAM serum level curves were extrapolated by means of trend lines in Excel 2010 software (Microsoft, Redmond, WA). According to distribution, the Wilcoxon test or the paired sample *t* test were used to determine differences between AUCs of unformulated niacin and AUCs calculated under the application of microencapsulated niacin. Changes in systemic metabolic parameters during the study weeks were determined by the Friedman test and Wilcoxon test or by paired *t* test according to distribution. To determine significant

changes in the microbial composition under niacin intervention, GraphPad was used to perform over time repeated ANOVA and paired *t* test. Statistical significance was set at $P < 0.05$.

RESULTS

Niacin Status in Humans in Relation to Obesity, Type 2 Diabetes, and the Gut Microbiome

First, we examined the association of NA and NAM with the microbiome in relation to human obesity and type 2 diabetes in 511 subjects from our FoCus cohort (Table 1). In agreement with earlier reports (2–4), the gut microbial composition of obese individuals showed significantly lower α-diversity measures of genera ($P = 0.036$) and operational taxonomic unit ($P < 0.001$) as well as significantly lower *Bacteroidetes* abundance on the phylum level ($P = 0.027$) compared with lean subjects. In obese subjects, a low niacin intake was associated with both a reduced α-diversity ($\rho = 0.286$, $P = 0.001$) and a lower *Bacteroidetes* abundance ($\rho = 0.191$, $P = 0.026$). This was further supported by a significant correlation of low NAM serum levels with a reduced α-diversity in obesity ($\rho = 0.176$, $P = 0.032$). Of interest, these findings were only significant in insulin-resistant obese subjects without clinically manifest type 2 diabetes (Fig. 1), suggesting that a niacin-related microbiome intervention might be most promising in the prevention of rather than in the treatment of type 2 diabetes. In this respect, it has to be taken into account that several subjects in the group with type 2 diabetes were treated with metformin, which is known to influence the composition of the gut microbiome (16) and might therefore beneficially interfere with the niacin-microbiome interaction.

Development of Novel Delayed-Release Niacin Microcapsules and In Vitro Evaluation

Having found an association of a low niacin intake with adverse microbiome changes, we developed a microencapsulation procedure to deliver increasing amounts of NA or NAM into the ileocolonic region where most of the gut bacteria are localized. To detect differential effects, two separate microcapsules were engineered, containing NA or NAM (see RESEARCH DESIGN AND METHODS). These microcapsules were subsequently compared with free NA and NAM in two human intervention studies: *1*) a bioavailability study and *2*) a proof-of-concept and safety study, as described in the section below. After the coating process of the cores, the NA and NAM microcapsules were analyzed by SEM for the verification of a homogenously distributed coating material. As shown in Fig. 2*A* and *B*, the outer shellac coating formed a homogenous layer covering the complete capsule surface, indicating the encapsulated compounds were sufficiently protected. To evaluate the in vitro dissolution profile, NA and NAM microcapsules were exposed to simulated gastrointestinal fluids according to modified pharmaceutical standards (17). The dissolution profiles in Fig. 2*C* and *D* show gastric resistance of NA and NAM microcapsules for 1 h at pH 1.4 and 0.5 h at pH 4.5 (release <3%). Afterward, the whole amount of encapsulated NA or NAM was released at pH 7.4. In contrast to NA microcapsules, NAM microcapsules had already released ~35% after 2 h at pH 6.8.

Bioavailability Study in Healthy Human Subjects

In the bioavailability study, 20 volunteers received single doses of NA or NAM microcapsules, followed by blood sampling in defined time intervals for 12 h (60-min intervals for 8 h after ingestion and two additional blood samples after 10 and 12 h). The procedure was repeated with increasing doses of NA (30, 150, 300 mg) and NAM (900, 1,500, 3,000 mg), with washout phases of 6 days in between. The microcapsules showed open and empty coating shells after gastrointestinal passage, indicating release of the ingredients (Fig. 3*A*). The NAM serum levels in the bioavailability study showed a delayed release 4 h after oral ingestion, suggesting opening of the capsules in the ileocolonic region (Fig. 3*B*). Owing to the rapid metabolism of NA into NAM (18), only minimal fluctuations of NA serum levels could be observed in the NA group, but not a consistent and dose-dependent increase (data not shown). Of importance for both NA and NAM, even under the highest dose of the microencapsulated compounds (300 mg NA, 3,000 mg NAM), the serum concentrations were not significantly increased compared with the reference dose of 30 mg free NA or 900 mg free NAM. This finding clearly indicates that the delayed-release microcapsules deliver high amounts of NA or NAM to the microbiome without significant alterations in serum concentrations. This is important, because high systemic levels of NA are known to be associated with negative side effects such as facial flushing and liver dysfunction (19).

Proof-of-Concept and Safety Study in Healthy Human Subjects

In the proof-of-concept and safety study, we analyzed the microbiome and determined the systemic insulin sensitivity in 10 metabolically healthy volunteers receiving daily NA or NAM microcapsules

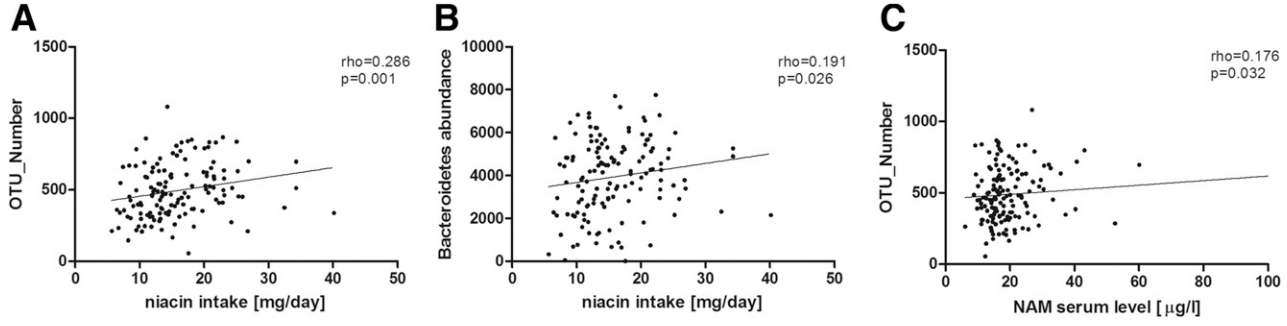

Figure 1—Niacin intake and serum levels in relation to the composition of the gut microbiome. Scatter plots and Spearman correlation analyses of niacin nutritional intake and α-diversity (*A*), niacin nutritional intake and *Bacteroidetes* abundance (*B*), and NAM serum levels and α-diversity of obese individuals without type 2 diabetes (*C*). OTU, operational taxonomic unit.

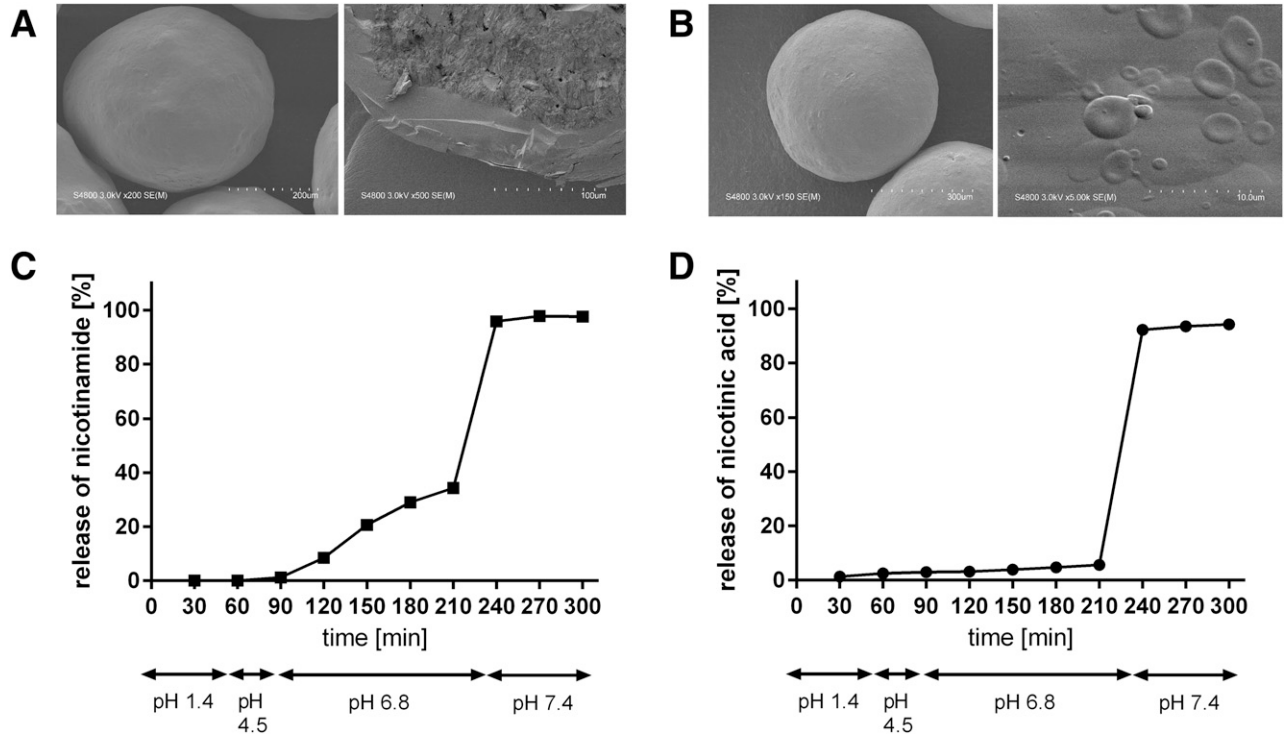

Figure 2—In vitro evaluation of novel delayed-release niacin microcapsules. *A*: SEM photographs show an undigested whole-coated NAM microcapsule (left), and a cross section of an undigested NAM microcapsule showing a tight coating with the NAM core inside (right). *B*: SEM photographs show an undigested whole-coated NA microcapsule (left) and the smooth coating surface of an undigested NA microcapsule at higher magnification (right). Results of in vitro release of NAM from NAM microcapsules (*C*) and NA from NA microcapsules (*D*) using pH change test. In vitro results are shown as mean ± SD (n = 3).

for 6 weeks, with a weekly increase in the dosage of NA (30 up to 300 mg) and NAM (900 up to 3,000 mg). A significant increase in *Bacteroidetes* abundance over the 6-week period was observed (P = 0.0025) in the NA group (Fig. 3*C*). In contrast, no significant change in the *Bacteroidetes* abundance was observed in the NAM group. This specificity of NA versus NAM is explained by the finding that *Bacteroidetes* are deficient in the enzymes nicotinamidase and nicotinamide phosphoribosyltransferase, resulting in the inability to metabolize NAM (20). Because obesity is associated with a reduction in *Bacteriodetes* abundance, these data suggest that NA is a stronger candidate for a targeted microbiome intervention than NAM. This is further supported by the systemic insulin sensitivity findings: biomarkers for insulin resistance were only significantly improved by microencapsulated NA but not by microencapsulated NAM. For example, microencapsulated NA, in contrast to free NA or any NAM formulation, induced a significant decrease in myostatin (1.78 ± 0.67 to 1.55 ± 0.48 ng/mL, $P < 0.05$) and fetuin-A levels (3.29 ± 1.10 to 2.66 ± 0.95 ng/mL, $P < 0.05$), which serve as markers for skeletal muscle and liver insulin resistance, respectively. Microencapsulated NA also resulted in a significant reduction of circulating osteopontin levels (2.77 ± 1.39 to 2.09 ± 0.78 ng/mL, $P < 0.05$), suggesting an improvement of metabolic inflammation, which is often found in individuals with prediabetes and diabetes. In terms of safety profile, one subject of the NA group experienced a mild elevation of aspartate aminotransferase levels at week 5, which returned to normal within 4 days. Apart from that, no safety signals or facial flushing occurred.

CONCLUSIONS

Several studies have reported the effect of the nutritional load (5) and dietary patterns (21) on the composition of the gut microbiome; however, to the best of our knowledge, our observation connecting niacin micronutrition and the human gut microbiome is novel. The positive correlations of niacin intake and niacin serum concentrations with α-diversity as well as the *Bacteroidetes* abundance suggest a favorable effect of niacin on the human gut microbial composition. We tested this hypothesis by developing delayed-release NA and NAM microcapsules to deliver increasing amounts of the micronutrients into the ileocolonic region. In vitro analyses showed that the NA and NAM microcapsule cores were protected by the coating layer (Fig. 2). Because of the ability of microcapsules (<2 mm) to pass the human pylorus independent of gastric emptying (22), the simulated gastric phase was reduced to 1 h. Both formulations released the entire amount of encapsulated niacin after reaching a pH of 7.4 (Fig. 2) because of the exceeded dissolution pH of shellac (pH 7.3) (23). This dissolution pH is in agreement with the postulated pH of the human ileum (24). However, the pH in the desired ileocolonic region can vary inter- and intraindividually and can be lower than the dissolution pH of shellac (25). Furthermore, a decreasing pH in the colon has been documented (26). A partial release at pH 6.8 is therefore desirable, as shown in Fig. 2*C*. Taking this information into account, our results showed a sufficient release of NA and NAM from our microcapsules and indicated a targeted release in the ileocolonic

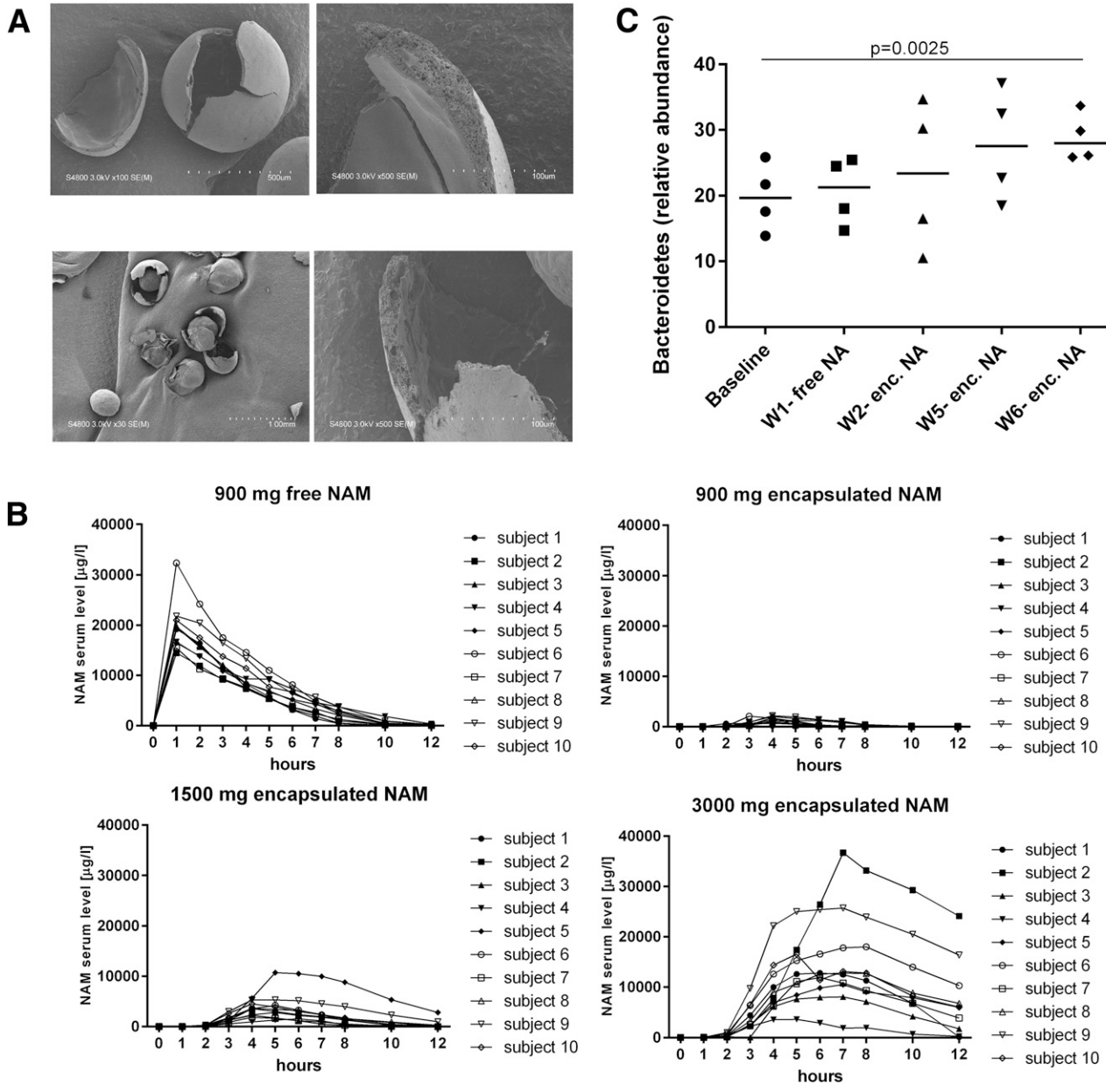

Figure 3—In vivo evaluation of novel delayed-release niacin microcapsules. *A*: SEM photographs show whole opened NAM microcapsules (top, left), porous and spongy surface and profile of NAM coating shell (top, right), whole opened NA microcapsules with the indigestible cellulose core inside (bottom, left), and porous and spongy surface and profile of opened NA microcapsules (bottom, right) after passage in the human volunteer. *B*: Pharmacokinetic curves of free NAM and different doses of microencapsulated NAM. *C*: Relative abundance of *Bacteroidetes* of the NA group in the course of the proof-of-concept and safety study.

region where the tested pH values were found.

As reviewed by Pišlar et al. (27) in 2015, the median time of gastric emptying after ingestion of nondisintegrating tablets is ~35 min, the small intestine transit time is 215 min (minimum–maximum 60–544), and the colon arrival time is 254 min (minimum–maximum 117–604). Thus our bioavailability curves with serum peak levels after 4−8 h indeed suggest a pH-dependent release of NA and NAM in the ileocolonic region. We note that the gastrointestinal transit times reported by Pišlar et al. (27) were examined in subjects who received the first meal at 4 h postdose, whereas meals given earlier can increase the time of gastric emptying or shorten small intestine transit time. During our bioavailability study, the first meal was given 3 h postdose. Nevertheless, the transit times still seem comparable to those reported by Pišlar et al. (27), because pellets smaller than 2 mm are emptied from the stomach very rapidly and are unaffected from the digestive state of the subject (22). A comparison of AUCs under dosage with microencapsulated and free NAM proved that no significant increase occurred in the total systemic niacin resorption. This finding clearly shows that our microencapsulation is able to deliver high amounts of NA/NAM into the colon without a significant increase in total systemic NA/NAM uptake compared with systemic uptake under application of the reference dose of free NA/NAM.

The dosage with our novel niacin formulations did not result in any severe

safety signals according to laboratory parameters, and no flush phenomenon or clinical symptoms were observed. In contrast, different niacin formulations to treat lipid disorders have been previously reported to induce several adverse effects. For instance, NA doses higher than 30−50 mg can induce facial flush, and doses of 300−2,000 mg NA can cause gastrointestinal symptoms (19,28). Further, administration of high NA doses can lead to liver disorders or even hepatitis and liver failure (19,28). Compared with NA, side effects of NAM are fewer and only appear with doses ≥3,000 mg (28,29). Of importance, side effects like flush or liver disorders also occurred with dosages with sustained- or extended-release formulations (30,31). However, these pharmacological niacin formulations contained much higher doses (>1,000 mg NA) and aimed a maximal systemic exposure to specifically treat lipid disorders. In complete contrast, our approach aimed for a topical exposure of NA/NAM in the colon, with a minimal increase in systemic resorption, to improve the gut microbial composition in obesity and type 2 diabetes. In summary, our data indicate that the developed NA and NAM microcapsules show a preferable safety profile, with no severe side effects and no systemic accumulation in healthy human volunteers.

In the current study, we found that the effect on the *Bacteroidetes* abundance and the systemic metabolism is specific to microencapsulated NA, whereas no effect was seen for any form of the NAM formulations. In this respect, it is important to mention that bacteria have been classified by virtue of their ability to synthesize NAD de novo from aspartic acid and/or the vitamin precursors NAM, NA, and NR (20). Therefore, the ability to use NAM was predicted on the basis of possession of homologs of nicotinamidase (*PncA*) and nicotinamide phosphoribosyltransferase (*NadV*). Remarkably, *Bacteroidetes* were reported to be deficient in both genes, resulting in the inability to metabolize NAM (20). This finding explains the specificity of the increase in *Bacteriodetes* abundance to the delayed-release NA intervention found in our study.

Note that the proof-of-concept and safety study reported here represents the equivalent of a phase 1 clinical trial and was therefore performed in healthy human volunteers showing no major insulin resistance (Supplementary Table 2). The HOMA index was normal in those subjects at the beginning and did not significantly change within the normal range during the intervention. However, we point out that this finding does not argue against an effect of microencapsulated NA on systemic insulin sensitivity, because independent researchers have shown that even a metformin therapy over a period of 6 months does not further improve a normal HOMA in nonobese subjects (32). In addition, the HOMA index is known not to detect early stages of insulin resistance; therefore, the emphasis has recently shifted toward myokines and hepatokines as alternative clinical biomarkers for skeletal muscle and liver insulin resistance (33). Myostatin is expressed in skeletal muscle (34) and is causally involved in muscle insulin resistance, because myostatin-null mice are protected from insulin resistance induced by diet-induced obesity (33). In humans, myostatin plasma levels have been shown to be strongly correlated to insulin resistance (34). On the hepatic site, fetuin-A was shown to be a natural inhibitor of insulin receptor tyrosine kinase (35) and associated with insulin resistance in human subjects (36). Of interest, strong associations of fetuin-A with the degree of insulin resistance were reported, especially in subjects without diabetes (37), making this hepatokine an interesting candidate for our proof-of-concept and safety study. Indeed, myostatin and fetuin-A levels were both significantly reduced by microencapsulated NA, suggesting beneficial effects on skeletal muscle and liver insulin resistance. Importantly, neither factor responded to free NA, indicating that the effect is most likely of an indirect nature, presumably via the beneficial changes of the microbiome. In addition to biomarkers for insulin sensitivity of skeletal muscle and liver, we measured osteopontin serum concentrations as a marker for adipose tissue inflammation (38). Osteopontin levels also decreased under microencapsulated NA, suggesting anti-inflammatory properties in addition to the beneficial metabolic effects (39).

In summary, in the current study we present evidence *1*) that the reduced α-diversity and *Bacteroidetes* abundance found in obese subjects is associated with a lower dietary niacin intake and *2*) that a gut-targeted NA supplementation by delayed-release microcapsules is able to beneficially affect the microbiome and the systemic insulin sensitivity.

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