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KLINISCHE STUDIE

Bacillus subtilis MM40[®] (DSM 21097) beim Metabolischen Syndrom

Darmbarriere, Glukose- & Lipidstoffwechsel, Darmmikrobiota

Studie zur Nahrungsergänzung (12 Wochen) · Placebokontrolliert, randomisiert

Medicom In Medical Center, Dnipro (Ukraine) · April 2026

70 Teilnehmer (Completer) mit Metabolischem Syndrom

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Bacillus subtilis MM40® (DSM 21097) beim Metabolischen Syndrom

Pathophysiologie · Rationale · Studienziel | April 2026

1) Metabolisches Syndrom & Pathophysiologie

- Metabolisches Syndrom (MetS): zentrale Adipositas, Insulinresistenz, Dyslipidämie, Hypertonie; deutlich erhöhtes Risiko für Diabetes mellitus Typ 2 und kardiovaskuläre Ereignisse.
- Gestörte Darmmikrobiota-Komposition, beeinträchtigte Darmbarrierefunktion (Zonulin) und reduzierte SCFA/Butyrat-Produktion gelten als pathophysiologische Verbindungsglieder zur metabolischen Dysregulation.
- Gezielte Probiotika – insbesondere sporenbildende Stämme wie Bacillus subtilis – zeigen Potenzial zur Modulation dieser Achsen; klinische Evidenz ist jedoch limitiert und stark stammspezifisch.

2) Zentrale pathophysiologische Kette bei MetS

- Dysbiose → ↓SCFA/Butyrat → Störung der Tight Junctions → ↑intestinale Permeabilität (↑Zonulin) →
- LPS-Translokation in die Zirkulation → chronische Low-grade-Entzündung (↑CRP) → Insulinresistenz (↑HOMA-IR) → Dyslipidämie & Hyperglykämie.
- Butyrat-Produzenten wie F. prausnitzii stabilisieren Epithelintegrität und stimulieren GLP-1-Sekretion (via FFAR); ihre Depletion ist ein Hallmark bei MetS und T2DM.

Drei Schlüsselmarker der MetS-Pathophysiologie

↓ SCFA

Darmbakterien-Metabolit
Butyrat, Acetat, Propionat
– Folge der Dysbiose

↑ Zonulin

Biomarker der
intestinalen Permeabilität
– „leaky gut“ bei MetS

↑ LPS

Endotoxin-Translokation →
Low-grade-Entzündung
(↑ CRP)

3) Rationale für B. subtilis MM40® & Studienziel

- Rationale: B. subtilis MM40® ist sporenbildend (hohe GI-Stabilität), moduliert Mikrobiota-Komposition und SCFA-Produktion; präklinische und limitierte klinische Daten zeigen Potenzial zur Reduktion der Permeabilität und Verbesserung der Glukose-Parameter.
- Studienziel: Wirkung von 12-wöchiger MM40®-Einnahme vs. Placebo auf Nüchternglukose, Insulin, HOMA-IR, Serum-Zonulin, Lipidprofil, CRP sowie fäkale Mikrobiota-Zusammensetzung bei Erwachsenen mit Metabolischem Syndrom.
- Gap: Humanstudien zu B. subtilis, die Glukosehomöostase, Darmbarriere und Mikrobiota gemeinsam bei MetS adressieren, sind bislang rar.

Studiendesign, Produkt, Teilnehmer & Messungen

Medicom In Medical Center, Dnipro (Ukraine) | April 2026

1) Studiendesign & Produktdetails

- Design: Placebokontrollierte, randomisierte Nahrungsergänzungsstudie; 12 Wochen (3 Monate) Intervention, 1× tägliche Einnahme.
- Produkt MM40®: B. subtilis DSM 21097 · Budapest Treaty Deposit DSM 33619 · EU-Marke Nr. 018279328 · Whole-Genome-Sequencing-bestätigt (CeGaT GmbH, Tübingen, 01/2022) · Hersteller: Magnat Vital GmbH.
- Dosierung: MM40® flüssig, 1× täglich 2 Tropfen (entspricht 5×10^8 KBE/Tag).
- Placebo: Kochsalzlösung NaCl 0,9 %, identische Farbe und Geschmack, ohne bakteriellen Gehalt.
- Ethik: Ethikkommission Medicom In Medical Center (Dnipro, Ukraine); Deklaration von Helsinki; schriftliche Einwilligung.

2) Teilnehmer, Ein- und Ausschlusskriterien

- Eingeschlossen: 72 Erwachsene (24 M / 48 F) mit MetS, randomisiert. 70 Completer mit vollständigen gepaarten Daten (37 Probiotik, 33 Placebo); 2 Dropouts.
- Einschluss: ≥ 3 MetS-Risikofaktoren (Nüchtern glukose $> 5,6$ mmol/L, erhöhter Blutdruck, Dyslipidämie, Taillenumfang > 94 cm M / > 80 cm F); Alter 18–65 Jahre; BMI > 25 kg/m².
- Ausschluss: MI/Stroke/Krebs < 12 Monate; diagnostizierter Diabetes oder andere endokrine Erkrankungen; Antibiotika/Probiotika-Einnahme < 3 Monate; GI-Erkrankungen.

Stichprobengrößen je Analyse-Ebene

n = 72

Randomisiert
24 M / 48 F
Baseline-Assessment

n = 70

Biochemische Analyse
37 Probiotik / 33 Placebo
Completer 12 Wochen

n = 38

Mikrobiota-Analyse
19 je Arm · Stuhlproben-
Einwilligung

3) Messungen & Mess-Methodik

- Biochemie: Nüchternblut Baseline & Woche 12; automatisierte klinische Chemie (ARCHITECT c4000, Abbott) für TC, HDL, LDL, TG, Glukose, Insulin, CRP. HOMA-IR = Glukose \times Insulin / 22,5.
- Zonulin: Serum-Zonulin via ELISA (Human Zonulin ELISA Kit, Invitrogen).
- Mikrobiota: 16S rRNA V3–V4 (Illumina MiSeq, paired-end); QIAamp DNA Stool Mini Kit (QIAGEN); QIIME2, SILVA (97 % OTU-Similarity).
- Statistik: Wilcoxon signed-rank (within-group), Mann–Whitney U (between-group); $p < 0,05$ signifikant.

Ausgangswerte der Teilnehmer bei Studienbeginn

Demografie · Anthropometrie · Biochemie · Zonulin | April 2026

1) Gruppen bei Baseline vergleichbar

- Die 70 Completer waren in allen demografischen, anthropometrischen und biochemischen Parametern zwischen Probiotik- und Placebo-Gruppe nicht signifikant unterschiedlich (Mann-Whitney U; $p > 0,05$ für alle Parameter).
- Trend-Abweichungen: Nüchtern glukose ($p = 0,06$) und HOMA-IR ($p = 0,07$) lagen grenzwertig höher in der Probiotik-Gruppe. Die HOMA-IR-Baseline-Imbalance wird in der Limitationen-Diskussion als möglicher Regression-to-the-Mean-Effekt berücksichtigt.
- Beide Gruppen erfüllten ≥ 3 MetS-Kriterien gemäß IDF/ATP-III-Konsens.

2) Demografische & anthropometrische Highlights

- Die Studienpopulation bestand überwiegend aus Frauen (67 %) im mittleren Lebensalter mit adipösem BMI und typischem MetS-Risikoprofil.
- Geschlechterverteilung: 24 M / 48 F (ausgewogen zwischen den Gruppen).

Zentrale Baseline-Werte im Überblick

~ 51 J.

Alter (Mittelwert)
Placebo 50,3 | Probiotik 51,9
 $p = 0,48$

~ 29

BMI (kg/m^2)
Placebo 29,2 | Probiotik 28,9
 $p = 0,86$

$\Delta 0,91$

HOMA-IR: 2,34 vs. 3,25
 $p = 0,07$ · grenzwertig
→ bei Interpretation beachten

3) Vollständige Baseline-Tabelle (Mittelwert \pm SD)

Parameter	Placebo (n=33)	Probiotik (n=37)	p
Alter (Jahre)	50,3 \pm 9,3	51,9 \pm 10,2	0,48
BMI (kg/m^2)	29,2 \pm 5,8	28,9 \pm 5,6	0,86
Nüchtern glukose (mmol/L)	5,23 \pm 0,62	5,84 \pm 1,03	0,06
Insulin ($\mu\text{U}/\text{mL}$)	9,94 \pm 7,34	12,42 \pm 6,59	0,14
HOMA-IR	2,34 \pm 1,80	3,25 \pm 1,87	0,07
Zonulin (ng/mL)	3,46 \pm 1,30	3,38 \pm 0,69	0,79
Gesamt-Cholesterin (mmol/L)	6,12 \pm 1,58	6,05 \pm 0,98	0,81
HDL (mmol/L)	1,53 \pm 0,45	1,38 \pm 0,43	0,19
LDL (mmol/L)	3,94 \pm 1,36	3,73 \pm 1,10	0,47
Triglyzeride (mmol/L)	1,57 \pm 0,93	1,72 \pm 0,92	0,54
CRP (mg/L)	1,92 \pm 1,52	1,91 \pm 1,76	0,98

Glukosehomöostase, Lipide, Entzündung & Barriere nach 12 Wochen

Δ-Werte, Signifikanzen & Einordnung | April 2026

1) Zentrale Effekte in der Probiotik-Gruppe

- Signifikante Verbesserungen in allen drei Hauptachsen: Glukosehomöostase (Glukose, Insulin, HOMA-IR), Lipidprofil (TC, LDL, HDL, TG) sowie systemische Low-grade-Entzündung (CRP).
- Darmbarriere: Zonulin-Reduktion innerhalb der Probiotik-Gruppe signifikant ($p = 0,0026$); Between-group-Differenz erreichte keine statistische Signifikanz ($p = 0,26$).
- Placebo-Gruppe: Keine signifikanten Verbesserungen; teilweise Verschlechterung (z. B. TC, CRP).

2) Einordnung in die Evidenzlage (Meta-Analysen)

- HOMA-IR-Reduktion -44% vs. typisch $10-20\%$ in publizierten Meta-Analysen zu Probiotika bei MetS/T2DM (Nikbakht 2018; Rittiphairoj 2021) → deutlich überlegener Effekt.
- Triglyzeride $\Delta -0,68$ mmol/L ($p = 0,00021$) — deutlich ausgeprägter Effekt gegenüber mehreren Probiotika-Studien, die keine Lipid-Effekte fanden (Shimizu 2015; Wu 2017).
- Mögliche Mechanismen: Bile-Salt-Hydrolase-Aktivität, Propionat-Produktion durch erhaltene *P. copri*. CRP-Reduktion (between $p = 0,006$) stützt entzündungshemmenden Effekt.

Drei Kern-Highlights (Probiotik-Gruppe nach 12 Wochen)

↓ 13 %

Nüchternglukose
5,84 → 5,08 mmol/L
within $p = 0,00049$ · between $p < 0,001$

↓ 44 %

HOMA-IR (Insulinresistenz)
3,25 → 1,82
within $p = 0,0001$ · between $p = 0,003$

↓ 39 %

CRP (Entzündung)
1,91 → 1,15 mg/L
within $p = 0,012$ · between $p = 0,006$

3) Vollständige Ergebnis-Tabelle: Δ nach 12 Wochen

Parameter	Δ Probiotik	Δ Placebo	within-p	between-p
Nüchternglukose (mmol/L)	-0,76	+0,21	0,00049	<0,001
Insulin (μU/mL)	-4,57	-0,28	0,0011	0,015
HOMA-IR	-1,44 (-44 %)	+0,08	0,0001	0,003
Gesamt-Cholesterin (mmol/L)	-0,48	+0,41	0,008	0,00018
HDL (mmol/L)	+0,35	+0,03	0,005	0,035
LDL (mmol/L)	-0,49	+0,26	0,006	0,002
Triglyzeride (mmol/L)	-0,68	-0,14	0,00021	0,009
CRP (mg/L)	-0,75	+0,89	0,012	0,006
Zonulin (ng/mL)	-0,55	-0,14	0,0026	0,26 (n.s.)

Darmmikrobiota-Veränderungen & mechanistische Wirkachse (n = 38)

Mikrobiota-Zusammensetzung nach 12 Wochen (Family- & Species-Level)

↑ Stabilisiert unter MM40®

F. prausnitzii ↑ 3,60 → 3,85 % ($p = 0,005$)
R. alkallicellulosi ↑ 0,39 → 0,65 % ($p = 0,0009$)
P. copri erhalten (6,97 → 5,91 %; $p = 0,039$)
Lachnospiraceae & *Ruminococcaceae* stabil

↓ Verschlechtert unter Placebo

B. vulgatus ↑ 1,32 → 2,92 % ($\Delta p = 0,027$)
Enterococcaceae: 2,0 → 14,8 % ↑↑
Lachnospiraceae 23 → 15 % · *Ruminococcaceae* 16 → 10 %
Prevotellaceae 7 → 4 % · *Enterobacteriaceae* 2 → 6 %

Family-Level: Probiotik-Gruppe stabil; Placebo mit markanten Shifts (99 Familien, Top 10 ≈ 80 %).

Interpretation: MM40® stabilisiert die Darmökologie bei MetS und verhindert die dysbiotische Verschiebung zu pro-inflammatorischen Taxa.

Mechanistische Wirkachse MM40® → Metabolische Verbesserung

- SCFA-Achse: Erhalt von Butyrat-Produzenten (*F. prausnitzii*, *R. alkallicellulosi*) → Butyrat ↑ → Tight-Junction-Stabilisierung (Zonulin ↓) → reduzierte LPS-Translokation → CRP ↓.
- GLP-1/FFAR-Signalweg: Butyrat aktiviert Free-Fatty-Acid-Rezeptoren, stimuliert GLP-1 → Insulin-Sensitivität ↑ → HOMA-IR ↓.
- Lipid-Modulation: Bile-Salt-Hydrolase-Aktivität + Propionat durch erhaltene *P. copri* → hepatische Lipogenese ↓ → TC, LDL, TG ↓; HDL ↑.
- Mikrobielle Stabilisierung: Verhinderung der Expansion von *Enterococcaceae* & *B. vulgatus* (pro-inflammatorisch, mit Insulinresistenz assoziiert).

Stärken der Studie

- Placebokontrolliert & randomisiert mit klinisch relevanten Endpunkten in 4 Domänen.
- Breites Wirkspektrum – Glukose, Lipide, Entzündung, Darmbarriere gleichzeitig.
- HOMA-IR-Reduktion (–44 %) übertrifft typische Probiotika-Meta-Analysen (10–20 %).
- Mikrobiota-Analyse mit zwei unabhängigen Tests (Post-Intervention + Δ) bestätigt.
- Konsistente Befunde über biochemische, immunologische, mikrobiologische Endpunkte.

Limitationen

- Kleine Stichprobe: biochemisch n = 70; Mikrobiom nur n = 38 – begrenzt Power.
- Single-Center-Design (Medicom In Medical Center, Dnipro) – populations-spezifische Bias möglich.
- Ernährung nicht objektiv überwacht; Variation kann Mikrobiota und Metabolik beeinflussen.
- 16S rRNA V3–V4 begrenzt Spezies-Auflösung; Metagenomik wäre genauer.
- HOMA-IR-Baseline-Imbalance (3,25 vs. 2,34; $p = 0,07$) → Regression-to-the-Mean möglich.
- 12-Wochen-Intervention – keine Daten zur Nachhaltigkeit nach Absetzen.

Zusammenfassung, Limitationen, Interessenkonflikte & Quelle

Fazit

Bei Erwachsenen mit MetS war die 12-wöchige MM40®-Einnahme (B. subtilis DSM 21097) vs. Placebo signifikant überlegen:

- Glukose -13 %, Insulin -37 %, HOMA-IR -44 % (alle between $p < 0,02$)
- Lipide: TC ↓, LDL ↓, TG -0,68 mmol/L ($p = 0,00021$), HDL ↑
- CRP -39 % (between $p = 0,006$); Zonulin within ↓ ($p = 0,0026$)
- Mikrobiota stabil: Erhalt F. prausnitzii, R. alkaliphilus; unter Placebo dysbiotisch

HOMA-IR-Reduktion (-44 %) übertrifft Probiotika-Meta-Analysen (10–20 %) deutlich.

MM40® positioniert sich als adjuvantes Nahrungsergänzungsprodukt beim MetS; größere multizentrische Bestätigungsstudien empfohlen.

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Bacillus subtilis supplementation in metabolic syndrome: effects on gut barrier function, glucose and lipid metabolism, and fecal microbiota composition. 2026.



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ORIGINALDOKUMENT

Englischsprachiges Originaldokument

Der folgende Abschnitt enthält das Originaldokument der Studie.

Personenbezogene Daten Dritter wurden unkenntlich gemacht.

Vogel W, Minch S, Prydyus I, Kryzhanovska N.

Bacillus subtilis Supplementation in Metabolic Syndrome: Effects on Gut Barrier Function, Glucose and Lipid Metabolism, and Fecal Microbiota Composition.

2026

Bacillus subtilis supplementation in metabolic syndrome: effects on gut barrier function, glucose and lipid metabolism, and fecal microbiota composition

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Abstract

Background & Aims: Gut microbiota dysbiosis contributes to the pathogenesis of metabolic syndrome (MetS) through impaired intestinal barrier function and reduced short-chain fatty acid (SCFA) production. *Bacillus subtilis*, a spore-forming probiotic, has shown potential to modulate gut microbiota and metabolic parameters, but clinical evidence remains limited. This study aimed to investigate the effects of 12-week dietary supplementation with *Bacillus subtilis* MM40® on metabolic parameters, intestinal permeability, and fecal microbiota composition in adults with MetS.

Methods: In a dietary supplementation study, 72 adults with MetS were assigned to receive *B. subtilis* MM40® (5×10⁸ CFU/day in liquid form) or placebo for 12 weeks (n = 70 completers). Fasting blood samples were analyzed for glucose, insulin, lipid profile, C-reactive protein (CRP), and serum zonulin. Fecal microbiota composition was assessed by 16S rRNA gene sequencing.

Results: The probiotic group showed significant reductions in fasting glucose (Δ -0.76 mmol/L; p = 0.00049), HOMA-IR (Δ -1.44; p = 0.0001), triglycerides (Δ -0.68 mmol/L; p = 0.00021), and CRP (Δ -0.75 mg/L; p = 0.012), with significant between-group differences for all metabolic parameters. Serum zonulin decreased within the probiotic group (p = 0.0026), although the between-group difference did not reach statistical significance. At the family level, the probiotic group maintained a stable microbiota composition, whereas the placebo group showed marked shifts including expansion of Enterococcaceae and loss of Lachnospiraceae and Ruminococcaceae. At the species level, beneficial taxa including *F. prausnitzii* (p = 0.005) and *R. alkalicellulosi* (p = 0.0009) were preserved in the probiotic group, while *B. vulgatus* expanded in the placebo group (Δ p = 0.027).

Conclusions: Twelve-week dietary supplementation with *B. subtilis* MM40® improved glucose homeostasis, lipid profile, and systemic inflammation, while maintaining a stable fecal microbiota composition in MetS patients. These findings support *B. subtilis* MM40® as a candidate adjunctive therapy for metabolic syndrome management.

Keywords:

Gut microbiota, *Bacillus subtilis*, intestinal permeability, glucose metabolism, lipid metabolism, metabolic syndrome

Introduction

Metabolic syndrome (MetS) is a cluster of interrelated cardiometabolic disturbances—central obesity, insulin resistance, dyslipidemia, and hypertension—that substantially increases the risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease [1–3]. Accumulating evidence implicates gut microbiota dysbiosis as a contributing factor in MetS pathogenesis. Depletion of beneficial taxa such as *Faecalibacterium prausnitzii*, *Roseburia* spp., and *Bifidobacterium* spp. leads to diminished production of short-chain fatty acids (SCFAs), particularly butyrate, which supports intestinal epithelial integrity, stimulates glucagon-like peptide-1 (GLP-1) secretion, and enhances peripheral insulin sensitivity [4–8].

Impaired intestinal barrier function represents a key mechanistic link between dysbiosis and systemic metabolic dysfunction. Disruption of epithelial tight junctions permits translocation of bacterial lipopolysaccharide (LPS) into the circulation, promoting chronic low-grade inflammation and insulin resistance [9,10]. Zonulin, the only known endogenous physiological modulator of intercellular tight junctions, has been validated as a biomarker of intestinal permeability, with elevated levels consistently reported in patients with obesity, T2DM, and MetS [11–13]. Probiotic supplementation has emerged as a promising strategy to counteract these disturbances: meta-analyses demonstrate that certain probiotic strains can modestly improve fasting glucose, HOMA-IR, and inflammatory markers, primarily through restoration of SCFA-producing communities and reinforcement of gut barrier integrity [14–17]. However, probiotic effects are highly strain-specific, necessitating targeted evaluation [18,19].

Among spore-forming probiotics, *Bacillus subtilis* is of particular interest owing to its exceptional gastrointestinal stability and capacity to modulate gut microbiota composition and SCFA production [20–22]. Preclinical and limited clinical data suggest that *B. subtilis* can reduce intestinal permeability and improve parameters of glucose metabolism [23,24]. Nevertheless, human studies specifically evaluating the combined effects of *B. subtilis* on glucose homeostasis, gut barrier function, and fecal microbiota composition in MetS patients remain scarce. The present study was designed to address this gap by investigating the effects of 12-week dietary supplementation with *Bacillus subtilis* MM40® (DSM 21097) on fasting glucose, insulin, HOMA-IR, serum zonulin, lipid profile, and fecal microbiota composition [25–27].

Materials and Methods

Study design

A dietary supplementation study was conducted over a 3-month intervention period. Prior to the intervention, participants were assigned to receive either *Bacillus subtilis* DSM 21097 (Budapest Treaty international deposit: DSM 33619; commercial name: *Bacillus subtilis* MM40®; EU trademark No. 018279328; strain identity confirmed by whole-genome sequencing, CeGaT GmbH, Tübingen; supplied by Magnat Vital GmbH; hereafter referred to as MM40®) in liquid form (dosage: 5×10^8 CFU/day) or a placebo (NaCl 0.9 % liquid of identical color and taste, without bacterial content) once daily for 3 months. Individuals were reminded to maintain their current physical activity levels and to continue on their usual diet throughout the study. Participants were also instructed to record any adverse events and to contact researchers if they stopped taking the supplement.

A simple randomisation schedule was manually prepared by an unblinded person who facilitated pre-screening and written informed consent. Participants who met the inclusion criteria were assigned a unique code number based on the randomisation schedule generated by the unblinded research member who was not involved in conducting the study assessment.

This study protocol was reviewed and approved by the Ethics Committee of Medicom In Medical Center (Dnipro, Ukraine). All procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to any data collection, including separate consent for fecal sample collection and microbiota analysis.

Participants

A total of 72 adults with metabolic syndrome were enrolled and randomized in the study, including 24 males and 48 females. Eligibility required the presence of at least three cardiometabolic risk factors consistent with metabolic syndrome, including elevated fasting glucose (>5.6 mmol/L), elevated blood pressure, dyslipidemia (low HDL cholesterol and/or elevated triglycerides), and increased waist circumference (>94 cm in men and >80 cm in women). Additional inclusion criteria were age between 18 and 65 years and BMI >25 kg/m². Participants were excluded if they had experienced myocardial infarction, stroke, or cancer within the previous 12 months, had diagnosed diabetes mellitus or other endocrine disorders, had used antibiotics or probiotics within the preceding 3 months, or had gastrointestinal disorders or were taking medications affecting gastrointestinal function. Of the 72 participants, 70 had complete paired data and were included in the final analysis, comprising 37 participants in the *Bacillus subtilis* MM40® group and 33 participants in the placebo group. Two participants were excluded from the final analysis due to incomplete post-intervention data.

Table 1. Baseline demographic, clinical, and laboratory characteristics of participants (mean \pm SD)

Parameter	Placebo (n=33)	Probiotic (n=37)	p-value
Age (years)	50.34 \pm 9.28	51.92 \pm 10.17	0.48
Total cholesterol (mmol/L)	6.12 \pm 1.58	6.05 \pm 0.98	0.81
HDL cholesterol (mmol/L)	1.53 \pm 0.45	1.38 \pm 0.43	0.19

LDL cholesterol (mmol/L)	3.94 ± 1.36	3.73 ± 1.10	0.47
Triglycerides (mmol/L)	1.57 ± 0.93	1.72 ± 0.92	0.54
C-reactive protein (mg/L)	1.92 ± 1.52	1.91 ± 1.76	0.98
Fasting glucose (mmol/L)	5.23 ± 0.62	5.84 ± 1.03	0.06
Insulin (μU/mL)	9.94 ± 7.34	12.42 ± 6.59	0.14
HOMA-IR	2.34 ± 1.80	3.25 ± 1.87	0.07
Zonulin (ng/mL)	3.46 ± 1.30	3.38 ± 0.69	0.79
BMI (kg/m ²)	29.15 ± 5.75	28.91 ± 5.61	0.86

Values are presented as mean ± SD. Between-group comparisons at baseline were performed using the Mann–Whitney U test for continuous variables. HOMA-IR was calculated as fasting glucose × fasting insulin / 22.5.

Measurements and analyses

Participants underwent fasting blood sampling at baseline and after 12 weeks of intervention. Blood samples were collected in the morning following an overnight fast and analyzed using standard automated clinical chemistry methods. Biochemical parameters were measured using an automated analyzer (ARCHITECT c4000 clinical chemistry analyzer, Abbott Laboratories, USA) in accordance with the manufacturer’s instructions. The assessed parameters included total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, fasting glucose, insulin, and C-reactive protein (CRP). Serum zonulin levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Zonulin ELISA Kit, Invitrogen, USA) according to the manufacturer’s protocol. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR), calculated as fasting glucose (mmol/L) multiplied by fasting insulin (μU/mL) divided by 22.5.

Fecal microbiota analysis

The composition of the gut microbiota was analyzed using 16S rRNA gene sequencing at baseline and after the 12-week intervention period. Fecal samples were self-collected by participants at home using sterile screw-cap containers provided by the study team. Participants were instructed to collect a walnut-sized stool sample in the morning prior to food intake, seal the container, and immediately place it in a provided insulated cooler bag with ice packs. Samples were transported to the laboratory within 4 hours of collection under controlled temperature conditions (2–8 °C) and stored at –80 °C until DNA extraction. Microbial DNA was extracted from fecal samples using a commercially available kit for stool DNA isolation (QIAamp DNA Stool Mini Kit, QIAGEN, Germany) according to the manufacturer’s instructions. Library preparation was performed using a standard amplicon-based protocol targeting the V3–V4 hypervariable regions of the bacterial 16S rRNA gene with primers compatible with the Illumina platform, and sequencing was carried out on the Illumina MiSeq platform using paired-end reads. Raw sequencing reads underwent quality control procedures, including removal of low-quality reads, trimming of sequences shorter than 150 base pairs, and exclusion of sequences containing ambiguous base calls. After quality filtering, sequences were dereplicated and denoised to generate operational taxonomic units (OTUs), and chimeric sequences were removed. Bioinformatic processing was performed using QIIME2. Taxonomic assignment was performed by comparison with the SILVA reference database using a 97% similarity threshold for OTU clustering.

Of the 70 participants included in the biochemical analysis, 38 (19 per arm) provided written consent for fecal sample collection and had complete paired stool samples available at both

baseline and week 12. Community-level microbiome analyses were performed on these 38 participants (76 individual samples across four study groups: Baseline Placebo, After Placebo, Baseline Probiotic, After Probiotic; $n = 19$ per group). Taxonomic profiles were summarized at the family level using relative abundance data derived from 16S rRNA amplicon sequencing to characterize overall compositional shifts between groups.

Statistical analysis

All biochemical analyses were performed using paired measurements obtained before and after the intervention. Microbiome analyses were conducted on paired fecal samples collected from the same 38 participants at baseline and after the 12-week intervention. Four species of clinical relevance were selected for targeted analysis: two beneficial SCFA-producing species (*Faecalibacterium prausnitzii* and *Ruminococcus alkalicellulosi*) and two species associated with unfavorable metabolic profiles (*Prevotella copri* [decline in placebo] and *Bacteroides vulgatus* [expansion in placebo]). For each species, post-intervention relative abundances were compared between the placebo and probiotic groups using the Mann–Whitney U test, and within-participant change-from-baseline scores (calculated from paired samples) were compared between groups. Continuous variables were expressed as mean \pm standard deviation (SD). Within-group comparisons of paired baseline and post-intervention measurements were performed using the Wilcoxon signed-rank test, and between-group differences in change-from-baseline values were assessed by comparing delta scores using the Mann–Whitney U test. All tests were two-sided, and a p -value <0.05 was considered statistically significant.

Results

Biochemical parameters

Biochemical parameters measured at baseline and after 12 weeks of intervention are presented in Table 2. At baseline, no statistically significant differences were observed between the groups for any parameter (Table 1). No serious adverse events were reported in either group during the study period.

In the *Bacillus subtilis* MM40® group, fasting glucose decreased from 5.84 ± 1.03 mmol/L to 5.08 ± 0.71 mmol/L ($\Delta -0.76 \pm 1.26$; within-group $p = 0.00049$), whereas no change was observed in the placebo group (5.23 ± 0.62 to 5.44 ± 0.88 mmol/L; $p = 0.33$); the between-group difference in change from baseline was highly significant ($p = 0.00078$). Fasting insulin declined from 12.42 ± 6.59 to 7.85 ± 4.33 μ U/mL in the probiotic group ($\Delta -4.57 \pm 7.68$; $p = 0.0011$) with no significant change in the placebo group ($\Delta -0.28 \pm 7.57$; $p = 0.50$; between-group $p = 0.015$). HOMA-IR showed a corresponding reduction in the probiotic group from 3.25 ± 1.87 to 1.82 ± 1.12 ($\Delta -1.44 \pm 2.08$; $p = 0.0001$), representing a 44% decrease, while the placebo group remained unchanged (between-group $p = 0.0026$).

Serum zonulin, a marker of intestinal permeability, decreased significantly in the probiotic group from 3.38 ± 0.69 to 2.83 ± 0.54 ng/mL ($\Delta -0.55 \pm 0.93$; $p = 0.0026$) but did not change in the placebo group (3.46 ± 1.30 to 3.30 ± 0.86 ng/mL; $\Delta -0.14 \pm 1.41$; $p = 0.32$); the between-group difference did not reach statistical significance ($p = 0.26$). Lipid parameters showed significant between-group differences for all measured fractions. Total cholesterol decreased in the probiotic group ($\Delta -0.48 \pm 1.07$ mmol/L; $p = 0.008$) while increasing in the placebo group ($\Delta +0.41 \pm$

0.86; $p = 0.009$; between-group $p = 0.00018$). HDL cholesterol increased significantly in the probiotic group ($\Delta +0.35 \pm 0.65$; $p = 0.005$) with no change in the placebo group (between-group $p = 0.035$). LDL cholesterol decreased in the probiotic group ($\Delta -0.49 \pm 1.01$; $p = 0.006$) while trending upward in the placebo group (between-group $p = 0.002$). Triglycerides showed the most pronounced reduction in the probiotic group ($\Delta -0.68 \pm 0.93$; $p = 0.00021$) compared with a modest non-significant decline in the placebo group ($\Delta -0.14 \pm 1.14$; $p = 0.70$; between-group $p = 0.009$). C-reactive protein decreased in the probiotic group ($\Delta -0.75 \pm 2.02$; $p = 0.012$) and increased in the placebo group ($\Delta +0.89 \pm 2.52$; $p = 0.07$), with a significant between-group difference ($p = 0.006$).

In summary, the probiotic group showed significant improvements across all measured metabolic parameters: glucose homeostasis (fasting glucose, insulin, and HOMA-IR), lipid profile (total cholesterol, HDL, LDL, and triglycerides), systemic inflammation (CRP), and intestinal barrier function (zonulin). The placebo group showed no significant improvements in glucose or lipid parameters over the same period.

Table 2. Changes in metabolic, inflammatory, and intestinal permeability markers after 12 weeks of supplementation with *Bacillus subtilis* MM40 or placebo

Parameter	BS base	BS w12	Change	p (within)	PL base	PL w12	Change	p (within)	p (btw)
Total chol (mmol/L)	6.05±0.98	5.57±1.29	-0.48±1.07	0.008	6.12±1.58	6.53±1.35	+0.41±0.86	0.009	0.00018
HDL (mmol/L)	1.38±0.43	1.72±0.49	+0.35±0.65	0.005	1.53±0.45	1.56±0.44	+0.03±0.60	0.70	0.035
LDL (mmol/L)	3.73±1.10	3.24±1.03	-0.49±1.01	0.006	3.94±1.36	4.20±1.28	+0.26±0.92	0.088	0.002
TG (mmol/L)	1.72±0.92	1.04±0.48	-0.68±0.93	<0.001	1.57±0.93	1.42±0.66	-0.14±1.14	0.70	0.009
CRP (mg/L)	1.91±1.76	1.15±0.67	-0.75±2.02	0.012	1.92±1.52	2.82±2.80	+0.89±2.52	0.068	0.006
Glucose (mmol/L)	5.84±1.03	5.08±0.71	-0.76±1.26	<0.001	5.23±0.62	5.44±0.88	+0.21±1.01	0.33	<0.001
Insulin (μU/mL)	12.42±6.59	7.85±4.33	-4.57±7.68	0.001	9.94±7.34	9.66±6.47	-0.28±7.57	0.50	0.015
HOMA-IR	3.25±1.87	1.82±1.12	-1.44±2.08	<0.001	2.34±1.80	2.41±1.84	+0.08±2.13	0.72	0.003
Zonulin (ng/mL)	3.38±0.69	2.83±0.54	-0.55±0.93	0.003	3.46±1.30	3.30±0.86	-0.14±1.41	0.32	0.26

Values are presented as mean \pm SD. Within-group p values were calculated using the Wilcoxon signed-rank test. Between-group p values were calculated using the Mann–Whitney U test on change-from-baseline scores. HOMA-IR = fasting glucose \times fasting insulin / 22.5. BS = *Bacillus subtilis* group ($n=37$); PL = Placebo group ($n=33$).

Family-level microbiota composition

Family-level taxonomic profiling revealed 99 bacterial families across all samples. The ten most abundant families accounted for approximately 80% of the total community in each group (Figure 2). At baseline, both groups exhibited comparable profiles dominated by Lachnospiraceae (23.2% in placebo, 27.2% in probiotic), Ruminococcaceae (16.2% and 16.8%), Bacteroidaceae (8.0% and 11.7%), and Prevotellaceae (7.1% and 8.0%). After 12 weeks, the placebo group showed marked compositional shifts: Lachnospiraceae declined from 23.2% to 15.0%, Ruminococcaceae from 16.2% to 9.8%, and Prevotellaceae from 7.1% to 3.8%, whereas Enterococcaceae expanded from 2.0% to 14.8% and Enterobacteriaceae from 2.3% to 6.3%. In contrast, the probiotic group exhibited a more stable overall profile, with Lachnospiraceae declining modestly from 27.2% to 21.1% and Ruminococcaceae remaining virtually unchanged (16.8% to 16.8%).

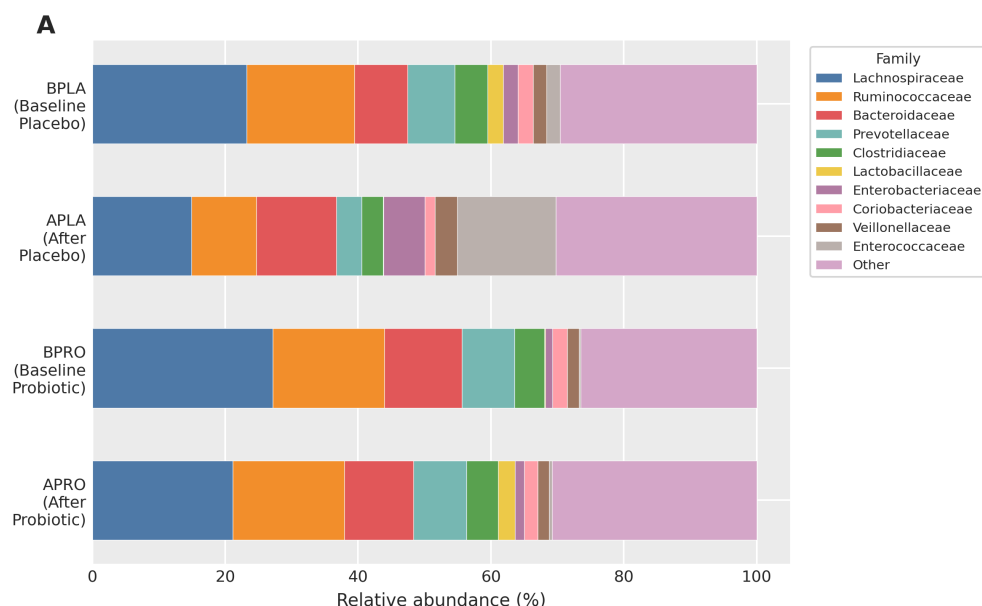


Figure 2. Family-level fecal microbiota composition in each study group.

Species-level changes in target taxa

Four species were selected for targeted analysis based on their documented roles in gut health and metabolic regulation (Figure 3). *Faecalibacterium prausnitzii*, the principal intestinal butyrate producer, was maintained in the probiotic group ($3.60 \pm 1.18\%$ to $3.85 \pm 2.04\%$) but declined in the placebo group ($3.64 \pm 1.81\%$ to $1.95 \pm 1.98\%$; post-intervention $p = 0.005$; $\Delta p = 0.023$). *Ruminococcus alkallicellulosi*, a cellulolytic fiber fermenter, significantly increased in the probiotic group ($0.39 \pm 0.23\%$ to $0.65 \pm 0.39\%$) while declining in the placebo group ($0.39 \pm 0.17\%$ to $0.31 \pm 0.54\%$; post-intervention $p = 0.0009$; $\Delta p = 0.007$). *Prevotella copri* was preserved in the probiotic group ($6.97 \pm 6.34\%$ to $5.91 \pm 7.76\%$) but declined in the placebo group ($6.26 \pm 5.25\%$ to $2.75 \pm 7.39\%$; post-intervention $p = 0.039$). *Bacteroides vulgatus*, a commensal species associated with pro-inflammatory signaling at elevated levels, increased in the placebo group ($1.32 \pm 1.44\%$ to $2.92 \pm 3.85\%$) but decreased in the probiotic group ($2.71 \pm 1.89\%$ to $1.78 \pm 2.88\%$; change-from-baseline $p = 0.027$). These findings demonstrate that *Bacillus subtilis* MM40® supplementation preserved beneficial SCFA-producing species while restraining the expansion of pro-inflammatory taxa.

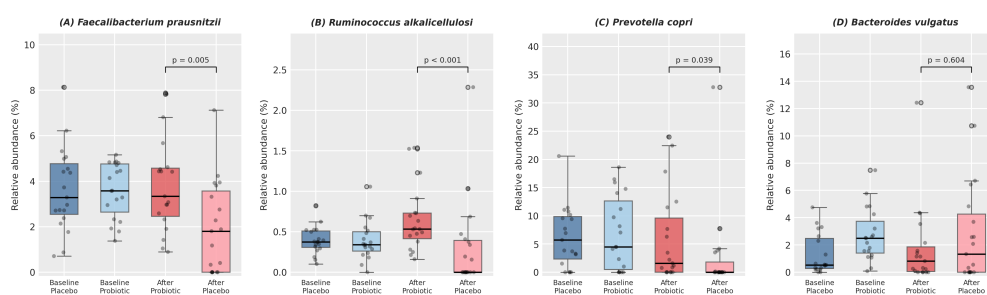


Figure 3. Species-level changes in selected fecal microbiota species at baseline and after 12 weeks. (A) *F. prausnitzii*, (B) *R. alkalicellulosi*, (C) *P. copri*, (D) *B. vulgatus*.

Discussion

This dietary supplementation study demonstrates that 12-week supplementation with *Bacillus subtilis* MM40® in patients with metabolic syndrome is associated with significant improvements in glucose homeostasis, lipid metabolism, systemic inflammation, and intestinal permeability markers, accompanied by preservation of beneficial gut microbiota composition. These findings extend the limited clinical evidence base for spore-forming probiotics in metabolic disease and provide mechanistic insights linking microbiota modulation to host metabolic outcomes.

Glucose homeostasis and insulin sensitivity

The observed reductions in fasting glucose (13%), fasting insulin (37%), and HOMA-IR (44%) in the probiotic group are clinically meaningful and exceed those reported in most meta-analyses of probiotic interventions in metabolic syndrome, which typically report HOMA-IR reductions of 10–20% [28,29]. The magnitude of the HOMA-IR reduction is notable and suggests that *B. subtilis* MM40® may have a potent insulin-sensitizing effect. The mechanistic basis for these improvements likely involves enhanced SCFA production by the preserved beneficial microbiota, as butyrate has been shown to improve insulin sensitivity through activation of free fatty acid receptors and stimulation of GLP-1 secretion [6,7].

Lipid metabolism and systemic inflammation

The significant improvements in lipid parameters, including reductions in total cholesterol, LDL, and triglycerides alongside an increase in HDL, contrast with several previous probiotic studies that reported no effects on lipid metabolism [30,31]. However, recent meta-analyses have identified strain-specific lipid-modifying effects among probiotics, particularly those that enhance bile salt hydrolase activity or SCFA-mediated regulation of hepatic lipogenesis [32,33]. The robust triglyceride reduction ($\Delta -0.68$ mmol/L, $p = 0.00021$) may be partially explained by the preserved abundance of *Prevotella copri*, which has been associated with improved carbohydrate and lipid metabolism through propionate production [34]. The concurrent reduction in CRP (between-group $p = 0.006$) supports the hypothesis that *B. subtilis* supplementation attenuates systemic low-grade inflammation, a central driver of metabolic syndrome pathophysiology [9,35].

Intestinal barrier function

The reduction in serum zonulin within the probiotic group ($p = 0.0026$) is consistent with previous reports linking *B. subtilis* supplementation to enhanced gut barrier integrity [23,36]. Although the between-group difference in zonulin change did not reach statistical significance ($p = 0.26$), the within-group reduction suggests a biologically relevant effect on tight junction regulation. Zonulin-mediated intestinal permeability has been implicated in the pathogenesis of insulin resistance through facilitation of endotoxin translocation [11,13], and the observed zonulin reduction may contribute to the improvements in glucose metabolism documented in this study.

Microbiota composition

At the family level, the probiotic group maintained a stable community composition, whereas the placebo group exhibited substantial rearrangement including loss of core SCFA-producing families (Lachnospiraceae, Ruminococcaceae) and expansion of Enterococcaceae. At the species level, the preservation of *F. prausnitzii* in the probiotic group is particularly noteworthy, as this species is the most abundant butyrate producer in the healthy human gut and its depletion is a hallmark of inflammatory and metabolic disorders [38,39]. *R. alkalicellulosi*, a cellulolytic fiber fermenter, also showed significant enrichment in the probiotic group, expanding the known range of taxa modulated by *B. subtilis* [40]. The reduction of *B. vulgatus* in the probiotic group is consistent with reports linking elevated levels of this species to pro-inflammatory signaling and insulin resistance [37].

Limitations

This study has several limitations. First, the relatively small sample size ($n = 70$) limits statistical power and generalizability. Second, the study was conducted at a single center, which may introduce population-specific biases. Third, dietary intake was not objectively monitored, and dietary variation may have influenced both metabolic parameters and microbiota composition. Fourth, 16S rRNA gene sequencing at the V3–V4 region provides limited species-level resolution for certain taxa, and metagenomic approaches would offer greater taxonomic and functional precision [41]. Additionally, the baseline imbalance in HOMA-IR between groups (3.25 vs. 2.34; $p = 0.07$), although not statistically significant, may have contributed to the magnitude of the observed HOMA-IR reduction through regression to the mean, and this should be considered when interpreting the glycemic findings. Finally, the 12-week intervention period does not address the sustainability of the observed effects after discontinuation of supplementation.

Despite these limitations, the consistency of the observed effects across multiple independent outcome domains—biochemical, immunological, and microbiological—strengthens the overall evidence for a genuine biological effect of *B. subtilis* MM40® supplementation in this patient population.

Conclusion

This dietary supplementation study demonstrates that 12-week supplementation with *Bacillus subtilis* MM40® (DSM 21097) significantly improves glucose homeostasis (fasting glucose, insulin, HOMA-IR), lipid metabolism (total cholesterol, HDL, LDL, triglycerides), and systemic inflammation (CRP) in patients with metabolic syndrome. The probiotic intervention was further associated with reduced serum zonulin levels within the probiotic group, although the between-group difference for zonulin did not reach significance, and with preservation of a stable fecal microbiota composition including beneficial SCFA-producing species such as *F. prausnitzii* and *R. alkalicellulosi*. These findings position *B. subtilis* MM40® as a promising adjunctive dietary supplement for the management of metabolic syndrome and warrant confirmation in larger, multicenter studies with extended follow-up periods and metagenomic characterization of the gut microbiota.

Acknowledgments

Bacillus subtilis MM40® (DSM 21097/DSM 33619) is a registered EU trademark (No. 018279328) owned by Magnat Vital GmbH. The probiotic strain used in this study was provided by the trademark holder. Strain identity of DSM 21097 and DSM 33619 was confirmed by whole-genome sequencing (CeGaT GmbH, Tübingen, January 2022).

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Conflict of Interest

S. Minch is employed by Magnat Vital GmbH, which supplied the *Bacillus subtilis* MM40® probiotic used in this study. All other authors declare no conflicts of interest.

Author Contributions

W. Vogel: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. S. Minch: Resources, Project administration, Writing – review & editing. I. Prydyus: Investigation, Methodology, Writing – review & editing. N. Kryzhanovska: Investigation (patient examination and clinical assessment), Data curation, Supervision, Writing – review & editing

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Figure Legends

Figure 2. Family-level fecal microbiota composition in each study group. Horizontal stacked bar chart showing the mean relative abundance (%) of the ten most abundant bacterial families and “Other” at baseline and after 12 weeks of intervention (n = 19 per group). BPLA, Baseline Placebo; APLA, After Placebo; BPRO, Baseline Probiotic; APRO, After Probiotic.

Figure 3. Species-level changes in selected fecal microbiota species at baseline and after 12 weeks of intervention. (A) *F. prausnitzii*, (B) *R. alkalicellulosi*, (C) *P. copri*, (D) *B. vulgatus*. Box plots show median, IQR, and whiskers extending to 1.5×IQR; individual data points overlaid. Mann–Whitney U test. n = 19 per group.