L-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans

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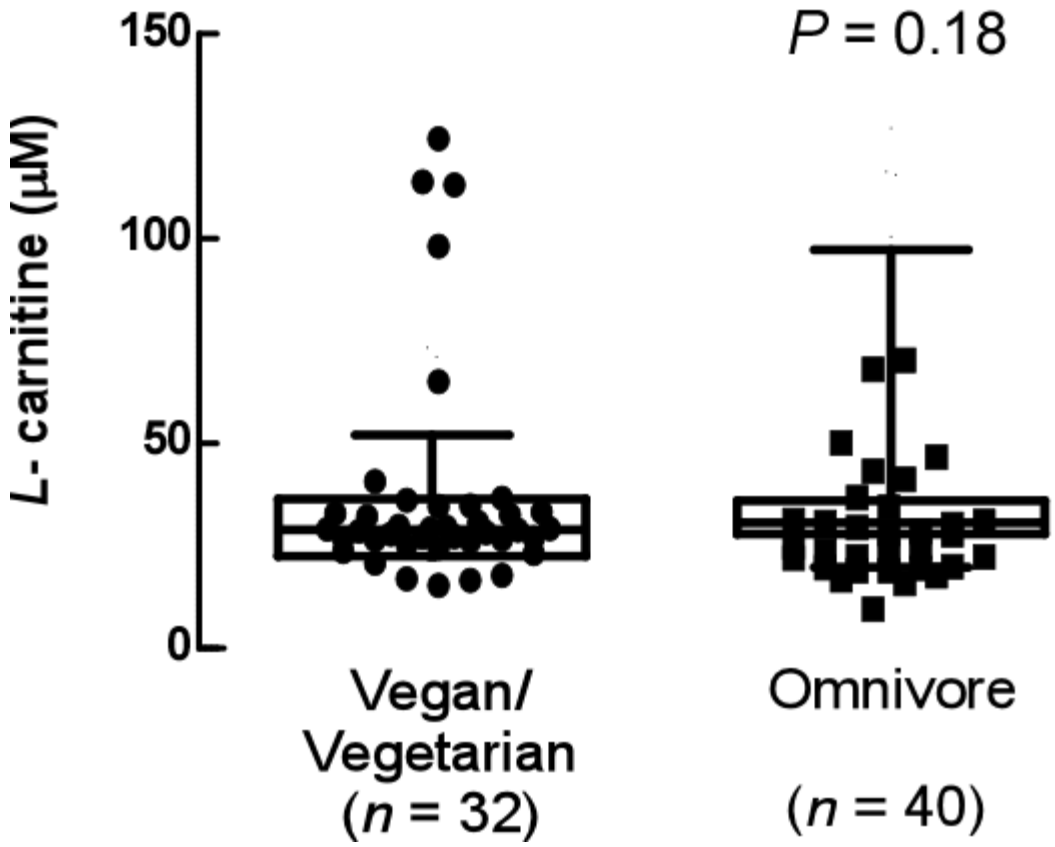
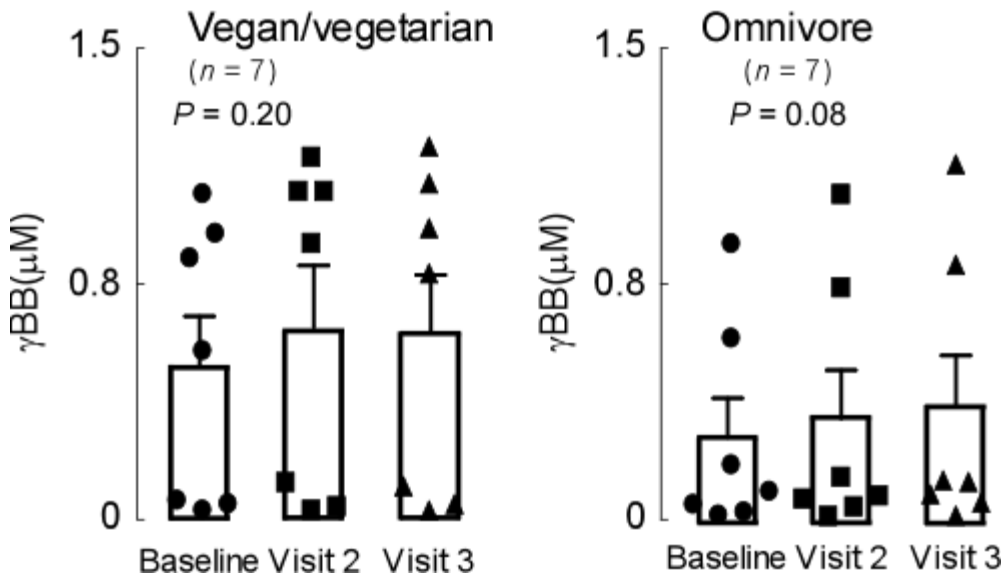
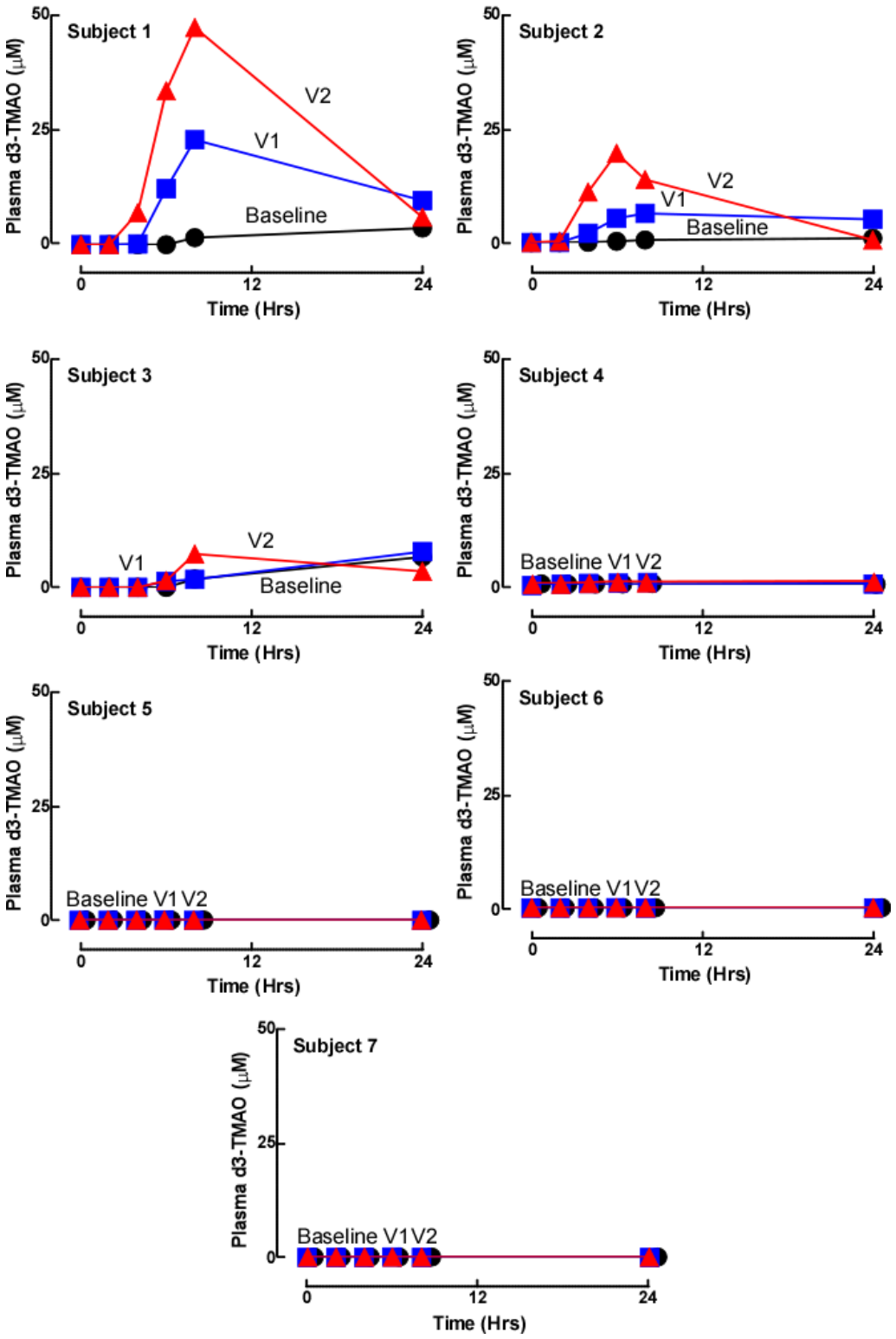
SUPPLEMENTARY DATA INDEX:



Supplemental Figure 1. Fasting plasma concentrations of *L*-carnitine in vegans and vegetarians (*n* = 32) versus omnivores (*n* = 40). Boxes represent the 25th, 50th, and 75th percentile and whiskers represent the 10th and 90th percentile. Plasma concentrations of *L*-carnitine were determined using LC/MS/MS. Wilcoxon rank sums was used to assess differences between groups.



Supplemental Figure 2. Plasma γBB concentrations in *L*-carnitine supplementation study. γBB plasma concentrations in subjects (*n* = 7 vegans/vegetarians and *n* = 7 omnivores) at baseline, and following daily *L*-carnitine supplementation at visit 2 (Visit 2 = 1 month), and visit 3 (Visit 3 = at least 2 months). Data presented as mean ± SEM. A repeated measures 1-way ANOVA test was used to assess differences among visits.



Supplemental Figure 3. Individual plots of plasma d3-TMAO from vegan and vegetarian subjects challenged with d3-*L*-carnitine at baseline (black circles), visit 1 (V1 = 1 month, blue squares), and visit 2 (V2 = 2-3 months, red triangles). Subjects are presented in decreasing magnitude of response of d3-TMAO production from d3-*L*-carnitine. Plasma concentrations of *L*-carnitine were determined using LC/MS/MS.

A

B

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Emergencia timonensis strain SN18 16S ribosomal RNA, partial sequence

Sequence ID: NR_144737.1 Length: 1480 Number of Matches: 1

Range 1: 1 to 516 GenBank Graphics Next Match Previous

Score	Expect	Identities	Gaps	Strand
942 bits(510)	0.0	514/516(99%)	0/516(0%)	Plus/Plus

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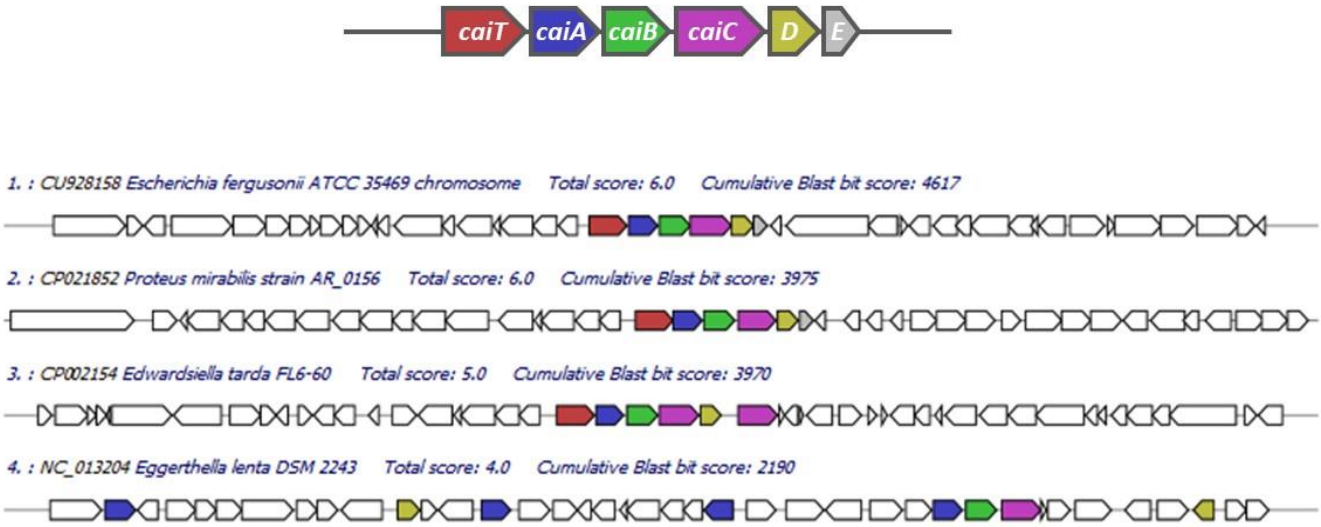
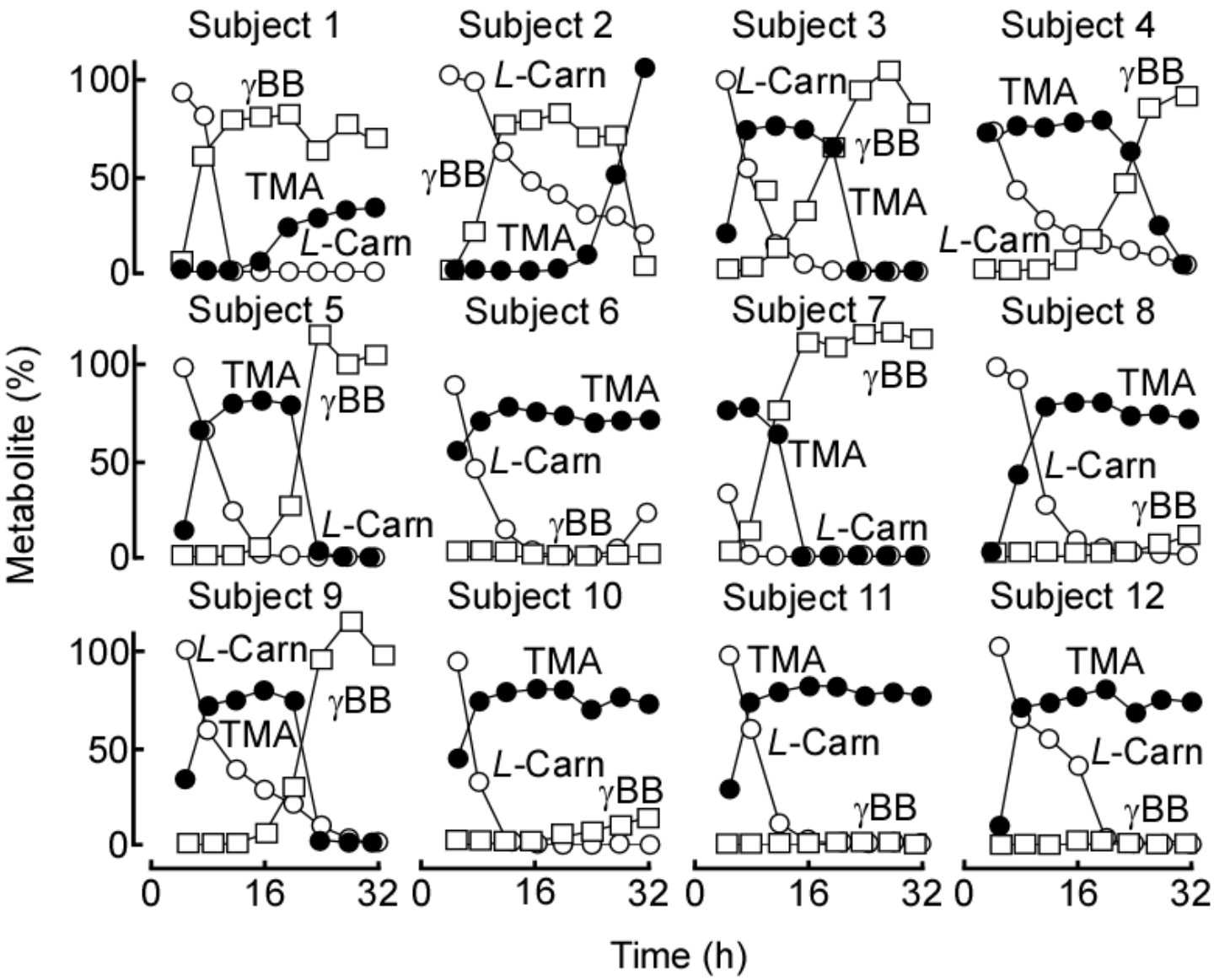
Supplemental Figure 4. (**A**) Characterization of SP2-71 revealed the presence of 4 microbes: *Hungatella hathewayi*, *Bacteroides dorei*, *Emergencia timonensis,* and *Peptoniphilus indolicus*. (**B**) 16S-rRNA Sequence alignment of strain SP2-71.3 from this study with *Emergencia timonensis* SN18 showing 99% sequence identity.



Supplemental Figure 5. Comparative genomics analysis of CaitTABCDE genes in organism found to utilize *L*-carnitine under anaerobic conditions.



Supplemental Figure 6. Anaerobic microbial *L*-carnitine catabolism generates γBB as an intermediate. Human fecal communities from 12 different donors including subjects presented in Figure 9B were studied for their *L*-carnitine → γBB → TMA transformation activity with sampling every 4 h for 32 h. Concentrations of *L*-carnitine (open circles), γBB (open squares) and TMA (filled circles) were determined by stable isotope dilution LC/MS/MS. Each point represents *n*=2 replicates.

Pool	Best match species*	Number of isolates	% of isolates	Isolates
SP1	*Peptoniphilus harei*	52	57%	1, 2, 3, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 19, 21, 23, 27, 28, 29, 30, 34, 36, 37, 38, 40, 43, 44, 45, 46, 47, 48, 50, 51, 52, 53, 55, 57, 59, 64, 67, 69, 70, 72, 80, 82, 83, 85, 86, 87, 89, 90, 100
SP2	*Clostridium hathewayi*	27	29%	4, 14, 18, 20, 22, 24, 25, 31, 35, 39, 41, 42, 49, 58, 63, 65, 66, 68, 71, 75, 78, 81, 88, 91, 92, 93, 94
SP3	*Clostridium hylemonae*	3	3%	32, 60, 74
SP4	*Bacteroides vulgatus*	2	2%	33, 54
SP5	no reliable identification	8	9%	56, 61, 62, 79, 84, 96, 97, 98

* best match from MALDI-TOF MS analysis using a BioTyper.

Supplemental Table 1. Microbial isolates comprising species pools for isolation of organisms involved in anaerobic *L*-carnitine catabolism.

Characteristics	Vegan/Vegetarian (*n*=10)	Omnivore (*n*=17)	*P*
Age (yrs)	43+ 18	43 +15	0.92
Sex (male, %)	50	47	0.88
Race			
Caucasian (%)	80	76	0.83
African American (%)	0	18	0.16
Hispanic (%)	10	0	0.18
Asian (%)	10	6	0.70
BMI (kg/m^2)	25 + 3.2	27+4	0.58
Comorbidities			
HPL (%)	10	29	0.24
HTN (%)	0	35	0.03
Hx of Diabetes (%)	0	6	0.43
Hx of cancer (%)	10	0	0.18
Hx of MI, stroke, PCI, CHF (%)	0	0	N/A
Medications			
Aspirin (%)	20	15	0.56
Beta blockers (%)	0	7	0.26
Statin (%)	10	29	0.22
ACEI/ARB (%)	0	12	0.26
Diuretic (%)	10	4	0.69
Calcium channel blocker (%)	10	6	0.69
Fish oil (%)	0	11	0.16

HPL= hyperlipidemia, HTN =hypertension, MI = Myocardial infarction, PCI=percutaneous coronary intervention, CHF=congestive heart failure, ACEI =angiotensin converting enzyme inhibitors, ARB = Angiotensin II receptor blockers

Supplemental Table 2: Baseline characteristics, comorbidities, and medications for subjects used in isotopologue challenge studies. Values represent means + SD or proportions expressed as a percentage (%) in the respective groups. Comparisons for means were completed using a Mann Whitney (Wilcoxon-Rank Sum test) and proportions were compared using a Pearson's chi-square test (X^2).

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	✓	2
		• Structured abstract recommended	✓	2
		• Information on target population or study sample	✓	2
Introduction				
Background	2	• Scientific background and explanation of rationale	✓	3-5
		• Theories used in designing behavioral interventions	✓	3-5
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	✓	19-21
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	✓	19-21
		• Recruitment setting	✓	19-21
		• Settings and locations where the data were collected	✓	19-21
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:	✓	19-21
		○ Content: what was given?	✓	19-21
		○ Delivery method: how was the content given?	✓	19-21
		○ Unit of delivery: how were the subjects grouped during delivery?	✓	19-21
		○ Deliverer: who delivered the intervention?	✓	19-21
		○ Setting: where was the intervention delivered?	✓	19-21
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	✓	19-21
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	✓	19-21
		○ Activities to increase compliance or adherence (e.g., incentives)	✓	20
Objectives	5	• Specific objectives and hypotheses	✓	3-5
Outcomes	6	• Clearly defined primary and secondary outcome measures	✓	19-21
		• Methods used to collect data and any methods used to enhance the quality of measurements	✓	19-21
		• Information on validated instruments such as psychometric and biometric properties	✓	19
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	✓	19-21
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	✓	19-21
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	✓	19-21
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	✓	19-21, 18

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	✓	20
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	✓	19-21
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	✓	6-14, 24
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	✓	24
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	✓	24
		• Methods for imputing missing data, if used	✓	24
		• Statistical software or programs used	✓	24
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	✓	19-21
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	✓	19-21
		○ Assignment: the numbers of participants assigned to a study condition	✓	19-21
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	✓	19-21
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	✓	19-21
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	✓	19-21
		• Description of protocol deviations from study as planned, along with reasons	✓	19-21
Recruitment	13	• Dates defining the periods of recruitment and follow-up	✓	19-21
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	✓	Supp Tbl 2
		• Baseline characteristics for each study condition relevant to specific disease prevention research	✓	Supp Tbl2
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	✓	Supp Tbl2
		• Comparison between study population at baseline and target population of interest	✓	Supp Tbl2
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	✓	Supp Tbl2

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	✓	19-21
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	✓	20
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	✓	6-14
		• Inclusion of null and negative findings	✓	6-14
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	✓	6-14
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	✓	19-21
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	✓	19-21
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	✓	14-18
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	✓	14-18
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	✓	14-18
		• Discussion of research, programmatic, or policy implications	✓	14-18
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	✓	18
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	✓	14-18

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/