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Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study

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Supplemental table 1: **Baseline Participant Characteristics**

Parameters	Placebo (n = 11)	Pasteurized (n=12)	Alive (n = 9)	P_{total}	*P1*	*P2*
Age (years)	49,45 ± 9,67	52,75 ± 7,16	52,89 ± 8,59	0,58[a]	*0,36*	*0,42*
Sex (nb (%))						
Male	5 (45)	4 (33)	6 (67)			
Female	6 (55)	8 (67)	3 (33)			
Height (cm)	173,05 ± 9,32	170,54 ± 8,8	172,11 ± 10,15	0,81[a]	*0,52*	*0,83*
Body Weight (kg)	112,45 ± 16,74	115,91 ± 17,23	109,03 ± 13,96	0,63[a]	*0,63*	*0,63*
BMI	37,63 ± 5,82	39,81 ± 4,77	36,82 ± 3,68	0,35[a]	*0,34*	*0,72*
Waist (cm)	120,2 ± 12,51	126,46 ± 8,75	120,06 ± 10,43	0,29[a]	*0,19*	*0,97*
Hip (cm)	118,8 (12,4)	123,46 (7,8)	117,83 (16,5)	0,22[b]	*0,13*	*0,65*
Waist-hip ratio	1,02 ± 0,11	1,03 ± 0,09	1,02 ± 0,10	0,97[a]	*0,82*	*0,94*
Systolic blood pressure (mm Hg)	150 (12)	150,00 (20)	140,00 (19)	0,15[b]	*0,38*	*0,18*
Diastolic blood pressure (mm Hg)	90,00 (9)	102,50 (19)	100 (19)	0,08[b]	*0,02*	*0,44*
Fasting Blood glucose (mg/dl)	100,33 (11,67)	103,00 (15,08)	94,33 (18,5)	0,34[b]	*0,24*	*0,82*
Glycated hemoglobin A1c (%)	5,53 ± 0,36	5,77 ± 0,42	5,63 ± 0,35	0,33[a]	*0,11*	*0,52*
Insulinemia (pmol/L)	111,36 (28,83)	155,83 (57,01)	128,7 (86,42)	0,030[b] *	*0,014**	*0,47*
% insulin sensibility	34,00 (8,5)	23,75 (9,17)	30,8 (21,65)	0,025[b] *	*0,007**	*0,5*
Total Cholesterol (mg/dl)	200,73 ± 43,19	203,00 ± 51,34	198,67 ± 20,47	0,97[a]	*0,91*	*0,9*
LDL Cholesterol (mg/dl)	131,63 ± 37,64	129,92 ± 41,10	128,78 ± 18,63	0,98[a]	*0,92*	*0,84*
HDL Cholesterol (mg/dl)	42 (7)	44,5 (12,25)	43 (18,5)	0,70[b]	*0,41*	*0,82*
Ratio (tot cholesterol/HDL)	4,65 ± 1,05	4,59 ± 1,14	4,84 ± 0,92	0,86[a]	*0,91*	*0,61*
Aspartate aminotransferase activity (U/L)	17,00 (8)	23 (17,75)	20 (11,5)	0,26[b]	*0,12*	*0,49*
Alanine aminotransferase activity (U/L)	21 (14)	37,5 (26,25)	30,78 (28,5)	0,14[b]	*0,42*	*0,34*
ɣ-Glutamyltransferase activity (U/L)	21 (17)	40,5 (51)	27 (5)	0,084[b]	*0,45*	*0,13*
Lactate dehydrogenase activity (UI/L)	166 (21)	197,5 (41,75)	180 (50)	0,082[b]	*0,023*	*0,46*
Creatine kinase (U/L)	90 (64)	96 (100)	111 (48,50)	0,68[b]	*0,48*	*0,33*
Triglycerides (mg/dl)	127,45 ± 46,49	139,83 ± 47,79	135,56 ± 44,13	0,84[a]	*0,58*	*0,69*
DDPIV activity (mU/ml)	14,72 (4,45)	15,58 (7,67)	13,89 (3,13)	0,12[b]	*0,36*	*0,18*

p_{total}: [a]P-values from one-way ANOVA except for Sex; [b]P-values from Kruskal-Wallis.
P1-2 = P-values for comparison of Placebo groups *versus* Pasteurized group (P1) and *versus* Alive group (P2) with the two-tailed Mann-Whitney U test or two-tailed unpaired T-test according to the distribution, with p > 0,017 due to the Bonferroni adjustment. Baseline values are presented as mean ± SD for normal variables while non-normal variables are presented as Median (inter-quartile range). *Significant difference between groups value (P < 0.05 for P_{total} ; P < 0.017 for P1 & P2)

Supplemental table 2: Safety parameters measured in all groups at baseline and after two weeks of treatment.

	Placebo		Pasteurized *Akk* -10^{10}		Alive *Akk* -10^{10}	
Safety parameters	**Baseline**	**Safety**	**Baseline**	**Safety**	**Baseline**	**Safety**
Systolic blood pressure (mm Hg)	145,92 ± 13,94	134,92 ± 18,53	150,38 ± 12,52	134,85 ± 19,20*	138,00 ± 17,97	138,21 ± 16,84
Diastolic blood pressure (mm Hg)	94,92 ± 13,97	90,31 ± 9,89	101,92 ± 11,28	91,77 ± 17,19	95,57 ± 19,29	92,43 ± 12,49
C-reactive protein (mg/dl)	5,23 ± 5,38	4,78 ± 3,14	7,77 ± 9,12	9,46 ± 11,33	4,07 ± 4,43	5,48 ± 5,860
White Blood cells (10^3/µl)	6,00 ± 1,08	6,78 ± 1,48*	6,47 ± 1,66	7,33 ± 1,54*	6,74 ± 1,28	7,5 ± 1,53*
Prothrombin time (sec)	11,48 ± 0,69	11,35 ± 0,62	11,66 ± 0,89	11,5 ± 0,83	11,47 ± 0,67	11,38 ± 0,70
Alanine aminotransferase activity (U/L)	23,38 ± 10,4	24,54 ± 10,94	47,62 ± 38,01	44,85 ± 35,59	33,43 ± 12,89	35,43 ± 13,44
Aspartate aminotransferase activity (U/L)	18,92 ± 5,78	18,77 ± 4,80	32,92 ± 31,45	35,23 ± 41,2	22,07 ± 7,08	23,64 ± 7,06
γ-Glutamyltransferase activity (U/L)	25,92 ± 16,59	29,38 ± 24,77	50,85 ± 32,93	47,85 ± 27,82	39,64 ± 21,1	40,86 ± 20,08
Urea (mg/dl)	33,69 ± 7,76	30,77 ± 6,76	30,38 ± 3,10	35,69 ± 13,38	32,21 ± 6,71	32,64 ± 7,33
Creatinine (mg/dl)	0,84 ± 0,15	0,83 ± 0,17	0,82 ± 0,13	0,84 ± 0,14	0,86 ± 0,14	0,90 ± 0,11
Glomerular filtration rate (ml min^{-1} $1,73m^{-2}$)	84,15 ± 16,09	86 ± 13,99	85,69 ± 14,13	82,92 ± 12,28	88,79 ± 15,84	82,14 ± 12,98
Creatine kinase activity (U/L)	114,3 ± 59,88	100,9 ± 58,96	152 ± 81,47	123,1 ± 63,19	118,4 ± 50,57	165 ± 175,8
Lactate dehydrogenase activity (UI/L)	177,4 ± 23,01	170,2 ± 21,46	193,9 ± 21,01	189,5 ± 39,34	190,9 ± 45,51	189,7 ± 32,22

This table includes all participants that proceeded to the safety visit: Placebo n=13, Pasteurized n=13, Alive n=14. Data are expressed as mean ± SD. Non-parametric two-tailed Wilcoxon matched-pairs signed ranks tests were performed to verify changes from baseline in each group.

* Significant difference from baseline value ($P < 0.05$)

Supplemental table 3: Proportion of subjects experiencing self-reported adverse effects after two weeks of treatment.

	Placebo	Pasteurized Akk - 10^{10}	Alive *Akk* - 10^{10}
Nausea	2/13	1/13	0/14
Flatulence	3/13	3/13	1/14
Bloating	2/13	0/13	1/14
Cramps	4/13	2/13	1/14
Borborygmi	3/13	1/13	3/14
Gastric reflux	2/13	1/13	0/14

Supplemental table 4: Safety parameters measured in all groups at baseline and at the end of the intervention.

	Placebo		Pasteurized *Akk* -10^{10}		Alive *Akk* -10^{10}	
Safety parameters	**Baseline**	**12 weeks**	**Baseline**	**12 weeks**	**Baseline**	**12 weeks**
Systolic blood pressure (mm Hg)	146,7 ± 15,60	129,00 ± 18,43*	151,08 ± 12,81	132,67 ± 11,79*	133,38 ± 12,41	124,63 ± 16,72*
Diastolic blood pressure (mm Hg)	91,00 ± 9,08	81,70 ± 9,04*	102,08 ± 11,77	83,92 ± 10,38*	92,25 ± 9,51	78,50 ± 13,03*
HbA1c (%)	5,53 ± 0,36	5,61 ± 0,30	5,77 ± 0,42	5,72 ± 0,37	5,63 ± 0,35	5,62 ± 0,33
C-reactive protein (mg/dl)	5,00 ± 5,51	5,31 ± 6,10	8,08 ± 9,45	8,84 ± 10,71	3,89 ± 4,08	3,78 ± 3,77
White Blood cells (10^3/µl)	6,14 ± 1,13	6,87 ± 1,81*	6,37 ± 2,43	6,24 ± 1,56	6,52 ± 2,40	6,74 ± 2,46
Prothrombin time (sec)	11,61 ± 0,69	11,37 ± 0,71	11,64 ± 4,61	11,61 ± 3,42	11,5 ± 0,73	11,61 ± 0,69
Alanine aminotransferase activity (U/L)	24 ± 11,26	23,36 ± 6,76	46,75 ± 39,56	39,92 ± 39,76	30,78 ± 13,84	30,89 ± 15,11
Aspartate aminotransferase activity (U/L)	18,82 ± 6,32	19,27 ± 3,64	33,33 ± 32,82	27,92 ± 26,21*	20,33 ± 5,66	20,33 ± 7,75
γ-Glutamyltransferase activity (U/L)	26,18 ± 17,93	29,55± 20,20	50,83 ± 34,39	42,33 ± 22,58*	34,11 ± 21,22	35,44 ± 33,00
Urea (mg/dl)	32,73 ± 7,99	32,36 ± 7,47	30,42 ± 3,23	33,42 ± 6,46	32,44 ± 6,58	33,56 ± 8,43
Creatinine (mg/dl)	0,84 ± 0,16	0,84 ± 0,18	0,81 ± 0,13	0,78 ± 0,09	0,83 ± 0,13	0,88 ± 0,11
Glomerular filtration rate (ml min^{-1} $1,73m^{-2}$)	86,27 ± 16,69	87,36 ± 18,50	84,00 ± 13,31	88,67 ± 11,60	91,44 ± 14,48	86,33 ± 12,99
Creatine kinase activity (U/L)	106,36 ± 48,71	110,09 ± 48,00	133,54 ± 85,66	106,09 ± 72,75*	117,89 ± 35,11	131,56 ± 70,39
Lactate dehydrogenase activity (UI/L)	173,46 ± 20,90	177,00 ± 38,99	197,17 ± 25,43	176,67 ± 27,13*	184,00 ± 24,98	176,78 ± 18,69

This table includes all participants that completed the study: Placebo n=11, Pasteurized n=12, Alive n=9. Data are expressed as mean ± SD. Non-parametric two-tailed Wilcoxon matched-pairs signed ranks tests were performed to verify changes from baseline in each group.
* Significant difference from baseline value ($P < 0.05$)

Supplemental table 5: Frequency of occurrence of adverse events during the whole intervention period.

Frequency %	Placebo	Pasteurized *Akk* - 10^{10}	Alive *Akk* - 10^{10}	*Ptotal*	*P1*	*P2*
Nausea	0,792	1,341	0,468	*0,196*	*0,726*	*0,653*
Flatulence	0,907	2,788	0,709	*0,319*	*0,436*	*0,798*
Bloating	1,204	0,185	0,356	*0,636*	*0,176*	*0,331*
Cramps	2,104	0,477	0,96	*0,375*	*0,065*	*0,256*
Borborygmi	0,593	0,651	1,194	*0,603*	*0,928*	*0,249*
Gastric reflux	0,798	2,480	0,121	*0,652*	*0,499*	*0,257*
Compliance %	99,4	99,08	99,76	*0,676*	0,56	0,3

Values are expressed as the percentage of events occurring for adverse events (frequency) and as the percentage of days where the packages were correctly taken (compliance).
p_{total} *: P*-values from one-way ANOVA.
P1-2: *P*-values for comparison of Placebo groups versus Pasteurized group (P1) and versus Alive group (P2) with the two-tailed unpaired T-test, with p > 0,017 due to the Bonferroni adjustment.