Supplements 1 to 4

Weight gain in anorexia nervosa does not ameliorate the faecal microbiota, branched chain fatty acid profiles, and gastrointestinal complaints

Isabelle Mack, Ph.D.[1], Ulrich Cuntz, M.D.[2], Claudia Grämer, Dipl. Nutrionist[2], Sabrina Niedermaier, Dipl. Nutrionist[2], Charlotte Pohl, cand. M.D.[1], Anderas Schwiertz, Ph.D.[3], Kurt Zimmermann, Ph.D [4], Stephan Zipfel, M.D.[1], Paul Enck, Ph.D.[1], John Penders, Ph.D.[5]

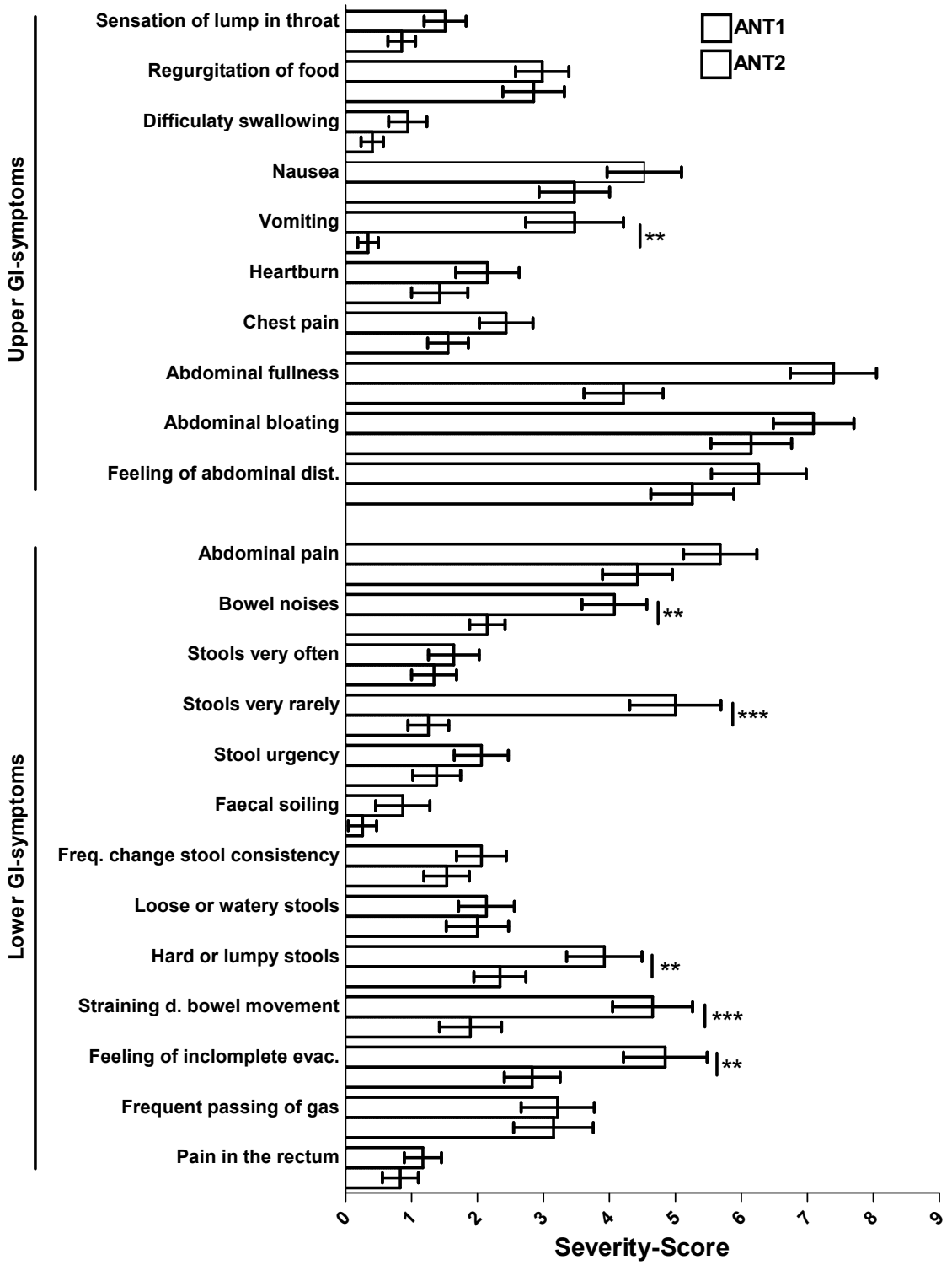
[1]Department of Psychosomatic Medicine and Psychotherapy, University Medical Hospital, Tübingen, Germany; [2]Klinik Roseneck, Center for Behavioral Medicine, Prien, Germany; [3]Institute of Microecology, Herborn, Germany; [4]SymbioPharm GmbH, Herborn, Germany; [5]Maastricht University Medical Center, NUTRIM School for Nutrition and Translational Research in Metabolism, Department of Medical Microbiology, Maastricht, The Netherlands

Supplement 1: Food consumption frequencies of anorexia nervosa patients (AN) before (T1) and after (T2) weight gain in comparison to normal-weight participants (NW).

	ANT1			ANT2			NW			FDR	FDR	FDR
Frequency per month	P25	Median	P75	P25	Median	P75	P25	Median	P75	ANT1 vs. NW	ANT2 vs. NW	ANT1 vs. ANT2
Milk	3	28	56	1	6	28	14	28	84		0.002	
Sugar-sweetened beverages	0	0	3	0	0	1	1	3	6	<0.001	<0.001	
Calorie-reduced beverages	0	6	70	0	1	6	0	0	3	<0.001		0.001
Fruit juice	0	1	6	0	1	3	5	14	35	<0.001	<0.001	
Vegetable juice	0	0	0	0	0	0	0	0	0			
Water	84	168	168	84	126	168	116	126	168			
Fruit and herbal tea	14	56	126	14	28	56	1	6	24	<0.001	<0.001	
Black tea	0	0	14	0	0	3	0	1	3			
Coffee	3	28	56	3	28	56	3	14	56			
Beer	0	0	1	0	0	0	0	1	6	<0.001	<0.001	0.037
Alcohol-free beer	0	0	0	0	0	0	0	0	0			
Wine, sparkling wine	0	0	3	0	0	1	1	3	6	<0.001	<0.001	
Cocktails	0	0	1	0	0	0	0	1	3	0.010		
Schnapps	0	0	1	0	0	0	0	1	1	0.029	0.004	
Corn flakes, cocao crispies	0	0	1	0	0	1	0	0	3			
Muesli, cereals	0	1	6	0	3	14	1	3	6			
Whole-meal bread or rolls	3	28	28	56	56	56	5	14	22		<0.001	<0.001
Brown bread	0	1	14	1	22	28	1	6	6		0.017	0.042
White bread, toast, rolls	0	3	14	6	22	28	3	6	14		0.004	0.011
Butter	0	3	25	56	56	56	5	18	56	0.01	<0.001	<0.001
Cream cheese	1	6	28	6	14	28	1	6	14		<0.001	
Cheese	1	6	25	14	28	56	5	6	14		<0.001	<0.001
Yoghurt, quark	3	14	28	14	22	28	3	6	14		<0.001	
Honey, marmelade, syrup	0	6	28	28	28	28	3	3	14		<0.001	<0.001
Hazelnut-chocolate spread	0	0	1	0	3	6	0	1	6	0.003		0.049
Eggs	0	1	3	3	6	6	2	3	6	<0.001		0.002
Poultry	0	3	6	0	3	6	1	3	6			
Doner kebab, hamburger	0	0	0	0	0	0	0	1	3	<0.001	<0.001	
Bratwurst/sausage	0	0	0	0	0	0	0	1	1	<0.001	<0.001	
Meat	0	1	3	0	6	6	1	3	6	0.044		0.008
Sausage, cold cuts	0	0	1	0	1	3	0	4	14	<0.001	0.004	
Ham	0	1	6	0	6	14	0	3	6			
Cold fish	0	1	3	0	3	6	0	0	3			
Cooked fish	0	3	6	3	6	6	0	2	3		<0.001	
Fresh fruit	6	28	56	14	14	28	14	28	56			
Cooked fruit	0	0	3	0	3	6	0	1	3		0.002	
Raw vegetables	6	28	56	14	14	28	14	22	28			0.011
Pulses	0	3	6	1	3	6	1	3	3			
Cooked vegetables	6	22	28	14	22	28	3	6	14	0.019	<0.001	
Pasta	1	3	6	6	6	6	6	6	14	<0.001		0.049
Rice	0	3	6	6	6	14	3	3	6		<0.001	0.019
Cooked potatoes	1	3	6	6	6	14	3	3	6		<0.001	0.001
Fried potatoes	0	0	1	0	1	3	0	1	1			0.002
Chips	0	0	0	0	0	0	1	1	3	<0.001	<0.001	
Pizza	0	0	1	0	1	3	1	1	3	<0.001	0.015	
Cake, muffins	0	1	10	3	6	14	3	3	6		0.011	
Biscuits	0	0	6	0	3	6	1	3	6			
Chocolate	0	1	14	3	6	14	3	6	14	0.003		
Sweets	0	6	28	0	3	18	1	3	14			
Ice cream	0	1	3	1	3	6	1	3	6	0.009		
Crisps	0	0	0	0	0	0	0	1	3	<0.001	<0.001	
Salty bicuits, salt sticks	0	0	3	0	0	1	0	1	1		0.008	
Nuts	0	1	4	0	1	3	0	2	3		0.055	

Legend: The median, P25 and P75 values for the food consumption frequencies within the last 28 days are presented. All p-values were false discovery rate (FDR)-adjusted. A FDR< 0.05 was considered as statistically significant. Annotation: The frequencies provide no information on the portion sizes consumed.

Supplement 2: Severity scores for gastrointestinal symptoms (GIS) in anorexia nervosa patients (AN) before (T1) and after (T2) weight gain.



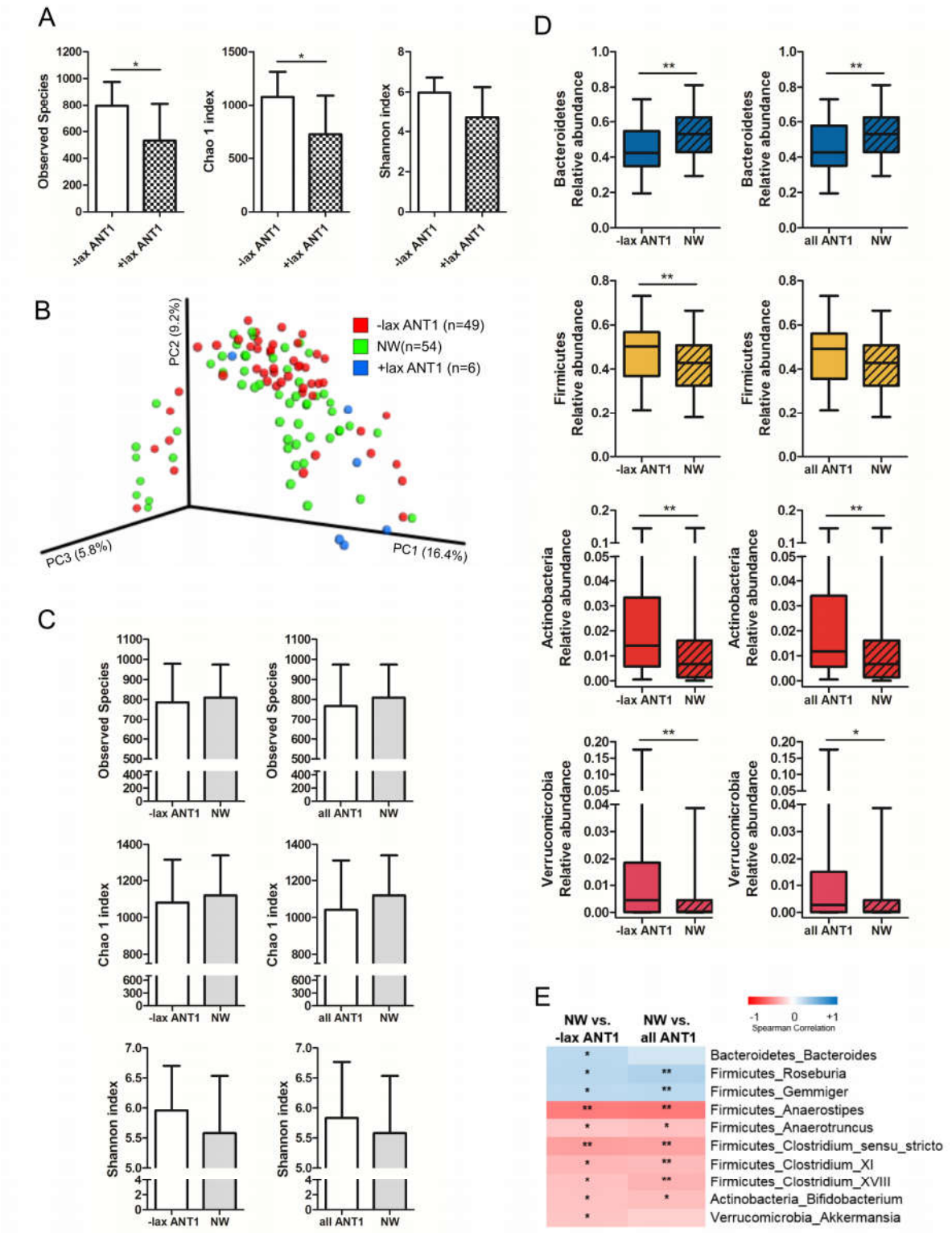
Legend: All differences of GIS between T1 and T2 in AN are given as mean±SE for clarity, although the data are not normally distributed. All p-values were false discovery rate (FDR)-adjusted. A FDR <0.05 was considered as statistically significant. ** indicates a FDR <0.01 and *** indicates a FDR <0.001.

Supplement 3: Correlations between SCFA and taxa at genus level for anorexia nervosa patients (AN) before (T1) and after (T2) weight gain and normal weight participants (NW).

Phylum_Genus	Acetate			Butyrate			Propionate			Valerate			Isovalerate			Isobutyrate		
	ANT1	ANT2	NW	ANT1	ANT2	NW	ANT1	ANT2	NW	ANT1	ANT2	NW	ANT1	ANT2	NW	ANT1	ANT2	NW
Actinobacteria_Bifidobacterium								-0,314										
Actinobacteria_Collinsella										***0,376***			0,304					
Actinobacteria_Eggerthella	-0,296	-0,311		-0,303	***-0,364***		-0,312				***-0,392***		-0,372	***-0,458***			-0,394	
Actinobacteria_Gordonibacter		-0,309			***-0,406***			***-0,422***										
Bacteroidetes_Bacteroides											***-0,418***						-0,37	
Bacteroidetes_Barnesiella									-0,275									
Bacteroidetes_Butyricimonas																0,312		0,333
Bacteroidetes_Paraprevotella									0,303									
Bacteroidetes_Prevotella											0,32							
Bacteroidetes_Alistipes										0,281								
Firmicutes_Lactococcus																		
Firmicutes_Streptococcus			0,298									***0,37***			0,338			***0,39***
Firmicutes_Clostridium_sensu_stricto		***0,417***			0,313			***0,394***		0,277	***0,451***			***0,426***			***0,444***	
Firmicutes_Blautia				0,29														
Firmicutes_Clostridium_XlVb																		
Firmicutes_Coprococcus				**0,429**											0,315			
Firmicutes_Dorea			0,274						0,354		***0,439***	***0,398***	**0,459**	0,324	0,326		***0,452***	***0,404***
Firmicutes_Lachnospiracea_incertae_sedis																		
Firmicutes_Roseburia	0,366	***0,425***		**0,453**	**0,535**	0,38	0,308	**0,486**						0,308				
Firmicutes_Clostridium_XI		0,325									***0,42***			***0,427***			***0,442***	
Firmicutes_Anaerotruncus	-0,346			**-0,435**			**-0,425**											
Firmicutes_Butyricicoccus		***0,407***			0,3290									0,324				
Firmicutes_Clostridium_IV							-0,294										0,328	
Firmicutes_Faecalibacterium		***0,375***			**0,451**			***0,405***										
Firmicutes_Flavonifractor				-0,306	-0,313								-0,373					
Firmicutes_Pseudoflavonifractor							-0,272											
Firmicutes_Ruminococcus					0,312													
Firmicutes_Sporobacter	-0,284						***-0,351***			0,281								
Firmicutes_Subdoligranulum																		
Firmicutes_Clostridium_XVIII										***-0,364***			-0,334					
Firmicutes_Holdemania																		
Firmicutes_Turicibacter				-0,282			-0,277				***0,384***			0,336			0,365	
Firmicutes_Phascolarctobacterium				***0,337***			0,277											
Firmicutes_Dialister																-0,273		
Proteobacteria_Oxalobacter																		
Proteobacteria_Parasutterella			-0,304			-0,271			-0,288						-0,271			
Proteobacteria_Sutterella																		
Proteobacteria_Bilophila		***-0,379***			-0,498			***-0,434***							0,298			0,289
Proteobacteria_EscherichiaShigella			0,315						0,378			***0,423***			0,345			**0,449**
Verrucomicrobia_Akkermansia							-0,286											

Legend: Spearman correlations between SCFA and bacterial genera that were present in at least 25% of the participants were conducted for all three groups separately. The p-values were false discovery rate (FDR) adjusted. A FDR<0.2 was considered as statistically significant. The table shows the correlation coefficients in bold if FDR<0.05, in bold italics if FDR>0.05 but <0.02 and in normal font if they did not withstand FDR adjustment.

Supplement 4: The role of laxative use in anorexia nervosa patients (AN) on the intestinal microbiota.



Legend (Suppl.4) **A:** Alpha diversity metrics are presented for AN patients without (-lax) and with a history (+lax) of laxative use before their inpatient stay (T1). **B:** Principal Coordinate Analysis (PCoA) of Bray-Curtis dissimilarities coloured according to –lax ANT1 (green), +lax ANT1 (blue) and normal-weight participants (NW; green). **C-E:** Sensitivity analysis: Alpha diversity metrics (**C**) and taxonomy at phylum (**D**) and genus level (**E**) are presented for NW versus ANT1 participants without a history of laxative use (-lax ANT1). In order to better interpret these sensitivity analysis, the data for NW versus ANT1 including patients with a history of laxative use (all AN, data from figures 3 and 5) are shown for comparison. P-values were adjusted for multiple testing (see methods). * indicates $p < 0.05$, except for D+E where after false discovery rate (FDR) adjustment a FDR <0.15 was considered as statistically significant in order to account for the beta error and where * indicates a FDR<0.15 and ** a FDR <0.05.