

## Supplementary Material

### **Severe, short-term sleep restriction reduces gut microbiota community richness but does not alter intestinal permeability in healthy young men**

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## Supplementary Material

**Supplementary Table S1.** Energy and macronutrient intakes and energy balance during three consecutive days of adequate sleep (AS) or sleep restriction (SR).

	<b>AS</b> <b>(mean ± SD)</b>	<b>SR</b> <b>(mean ± SD)</b>	<b>Mean difference</b> <b>[95% CI]</b>	<b>P-value<sup>†</sup></b>
Energy (kcal/d)	2762 ± 349	2877 ± 339	115 [67, 164]	<0.001
Carbohydrate (g/d)	432 ± 64	459 ± 64	27 [18, 35]	<0.001
Fat (g/d)	84 ± 10	85 ± 8	1 [-2, 3]	0.55
Protein (g/d)	68 ± 6	69 ± 7	1 [-1, 2]	0.36
Energy Balance (kcal/d)	73 ± 131	42 ± 106	-31 [-72, 10]	0.12

n = 19.

<sup>†</sup>Paired samples *t*-test.

## Supplementary Material

**Supplementary Table S2.** Morning fasted body weights measured during three consecutive days of adequate sleep (AS) or sleep restriction (SR).

	Day 1	Day 2	Day 3	Day 4	<i>P</i> -value <sup>†</sup>		
					Condition	Day	Condition* Day
<b>AS</b>	77.0 ± 7.2	76.9 ± 7.4	76.9 ± 7.4	76.9 ± 7.5	<0.001	0.71	0.22
<b>SR</b>	75.9 ± 7.6 <sup>a</sup>	76.4 ± 7.4 <sup>b</sup>	76.3 ± 7.3 <sup>b</sup>	76.4 ± 7.6 <sup>b</sup>			

<sup>†</sup>Linear mixed model with condition, day and their interaction as fixed factors and subject as a random intercept.

<sup>a,b</sup>Linear mixed model within condition, including day as a fixed factor and subject as a random intercept. Main effect of day within AS,  $P = 0.84$ . Main effect of day within SR,  $P = 0.02$ . Within a row, values not sharing a superscript letter are significantly different,  $P < 0.05$ .

## Supplementary Material

**Supplementary Table S3.** Serum markers of stress and inflammation following three consecutive nights of adequate sleep (AS) or sleep restriction (SR).

	<b>SR-day 1</b>	<b>SR-day 4<sup>†</sup></b>	<b>AS-day 4<sup>‡</sup></b>
Cortisol ( $\mu\text{g/dL}$ ; mean $\pm$ SD)	16.0 $\pm$ 2.4	13.5 $\pm$ 2.8***	15.5 $\pm$ 2.1*
hsCRP (mg/L; median [IQR]) <sup>§</sup>	0.34 [0.90]	0.25 [1.41]	0.39 [1.34]

n = 19.

<sup>†</sup>Within-condition comparisons analyzed by paired *t*-tests. \*\*\*Significantly different from SR-day 1 ( $P < 0.001$ ).

<sup>‡</sup>Between-condition comparisons analyzed by mixed model ANOVA. \*Significantly different from SR-day 4 ( $P = 0.03$ ).

<sup>§</sup>Log<sub>10</sub>-transformed for analysis.

## Supplementary Material

**Supplementary Table S4.** Intestinal permeability measured following three consecutive nights of adequate sleep (AS) or sleep restriction (SR).

	AS	SR	<i>P</i> -value <sup>†</sup>
Urine volume (mL; mean $\pm$ SD)	985 $\pm$ 359	1063 $\pm$ 249	0.53
Lactulose (%; median [IQR]) <sup>‡</sup>	0.08 [0.10]	0.10 [0.11]	0.77
Mannitol (%; median [IQR]) <sup>‡</sup>	16.3 [6.2]	15.6 [5.9]	0.47
Lactulose:mannitol ratio (median [IQR]) <sup>‡</sup>	0.0047 [0.0056]	0.0057 [0.0067]	0.39

n=17.

<sup>†</sup>Mixed model ANOVA.

<sup>‡</sup>Log<sub>10</sub>-transformed for analysis.

## Supplementary Material

**Supplementary Table S5.** Genera and amplicon sequencing variants (ASVs) demonstrating between-condition differences ( $P < 0.05$ ) following two consecutive days of adequate sleep (AS) or sleep restriction (SR).<sup>†</sup>

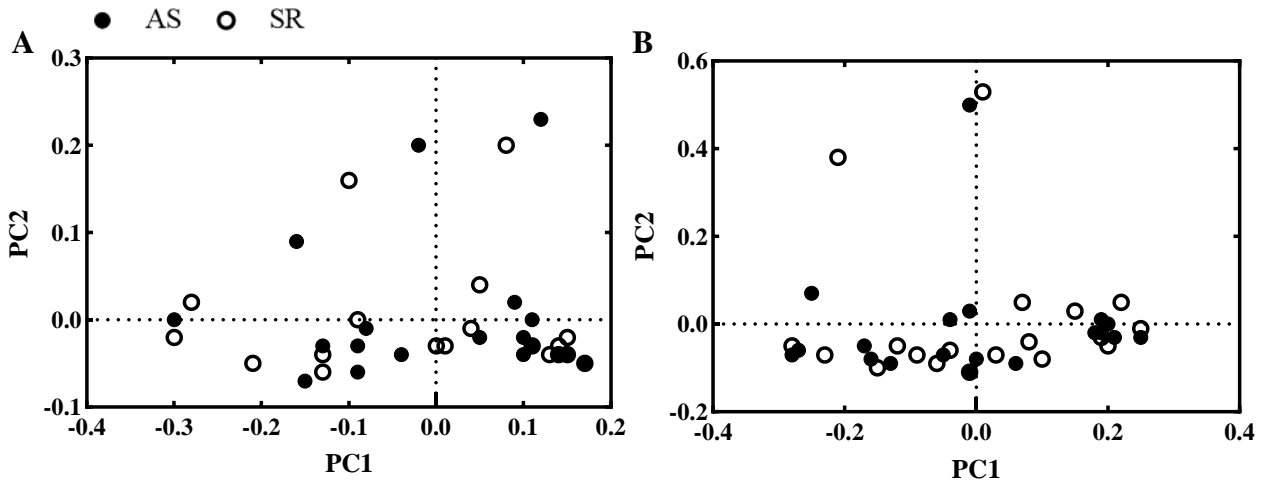
Taxon	$\beta \pm SE^{\ddagger}$	P-value	q-value
Genera			
<i>Campylobacter</i>	-0.32 ± 0.10	0.007	0.29
<i>Roseburia</i>	-0.17 ± 0.07	0.02	0.43
<i>Papillibacter</i>	0.26 ± 0.12	0.04	0.57
ASV			
Firmicutes.Clostridia.Clostridiales.Ruminococcaceae	-0.39 ± 0.10	0.001	0.20
Firmicutes.Clostridia.Clostridiales.Lachnospiraceae.	-0.34 ± 0.13	0.02	0.92
Clostridia.Clostridiales.Lachnospiraceae. <i>Clostridium</i>	-0.26 ± 0.10	0.02	0.92
Firmicutes.Clostridia.Clostridiales.Ruminococcaceae. <i>Subdoligranulum variabile</i>	-0.30 ± 0.11	0.02	0.92
Firmicutes.Clostridia.Clostridiales.Ruminococcaceae	-0.19 ± 0.08	0.03	0.92
Firmicutes.Clostridia.Clostridiales.Ruminococcaceae	-0.31 ± 0.13	0.03	0.92
Bacteroidetes.Bacteroidia.Bacteroidales.Bacteroidaceae. <i>Bacteroides</i>	0.25 ± 0.11	0.03	0.92
Bacteroidetes.Bacteroidia.Bacteroidales.Prevotellaceae. <i>Prevotella</i>	-0.12 ± 0.06	0.04	0.92
Firmicutes.Clostridia.Clostridiales.Lachnospiraceae. <i>Roseburia inulinivorans</i>	-0.14 ± 0.07	0.05	0.92

<sup>†</sup>Analyzed by Microbiome Multivariable Associations with Linear Models<sup>1</sup> with treatment, treatment order and study period included as fixed factors, and subject as a random factor.

<sup>‡</sup>AS is the reference group.

<sup>1</sup>Mallick H, Rahnavard A, McIver LJ, et al. Multivariable association discovery in population-scale meta-omics studies. *PLoS Comput Biol.* 2021;17(11):e1009442.

## Supplemental Figure 1



**Supplemental Figure 1. Gut microbiota composition following two consecutive days of adequate sleep (AS) or sleep restriction (SR).** Principal coordinates analysis of (A) weighted and (B) unweighted Unifrac distances. PERMANOVA,  $P = 1.0$ .