

# **Oral supplementation of sodium butyrate attenuates the progression of non-alcoholic steatohepatitis**

*Nutrients*

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**Table S1. Primer sequences used for real-time PCR.**

	<b>Forward (5'- 3')</b>	<b>Reverse (5'- 3')</b>
<i>18S</i>	gtaacccgttgaacccatt	ccatccaatcggtagtagcg
<i>αSma</i>	ctgacagaggcaccactgaa	catctccagagtccagcaca
<i>Aanat</i>	gcttctcctagtcacagacccc	agcggaaactcactggcaggg
<i>Hiomt</i>	ccctggcggtccaccttcctg	atggcgggtgaaggggtcgtc
<i>Il1β</i>	tggctgtggagaagctgtgg	gtccgacagcacagaggcttt
<i>Il6</i>	ccacgccttcctacttca	tgcaagtgcacatcgttggtc
<i>iNos</i>	cccctggaagtttctcttcaaagt	gattctggaacatctgtgctgtcc
<i>Mtr1a</i>	aatgccactcagcaggctccag	agcagggtgccagaatgtcca
<i>Mtr1b</i>	agggtaccgtgcctgtcaa	aggtttgctgctaggccact
<i>Myd88</i>	caaaagtggggtgcctttgc	aaatccacagtgtccccaga
<i>Tgfβ</i>	gtctgggaccctgccctat	ttgcaggagcgcacaatcat
<i>Tlr4</i>	agccattgctgccaacatca	gctgcctcagcagggacttc
<i>Tnfa</i>	cagccaaccaggcagcgttcct	cctgccacaagcaggaatga

αSma, alpha smooth muscle actin; Aanat, serotonin N-acetyltransferase; Hiomt, hydroxyindole-O-methyltransferase; Il, interleukin; iNos, inducible nitric oxide synthase; Mtr, melatonin receptor; Myd88, myeloid differentiation primary response gene 88; Tgfβ, transforming growth factor beta; Tlr4, toll-like receptor 4; Tnfa, tumor necrosis factor alpha. Expressions were normalized to 18S mRNA expression.

**Table S2. Effect of an oral supplementation of SoB on fibrosis markers in livers of mice with FFC-induced NASH.**

	Diet groups				<i>p</i> (two-way ANOVA)		
	C	FFC	C + SoB	FFC + SoB	DExSoBE	SoBE	DE
<b>Sirius red staining</b> (% of microscopic field)	1.2 ± 0.2 <sup>a</sup>	1.5 ± 0.4 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>	1.1 ± 0.1 <sup>a</sup>	>0.05	>0.05	>0.05
<b><i>αSma</i> mRNA expression</b> (% of control)	100.0 ± 13.5 <sup>a</sup>	177.5 ± 47.8 <sup>a</sup>	192.1 ± 57.1 <sup>a</sup>	262.3 ± 81.5 <sup>a</sup>	>0.05	>0.05	>0.05
<b><i>Tgfb</i> mRNA expression</b> (% of control)	100.0 ± 15.4 <sup>a</sup>	129.8 ± 29.4 <sup>a</sup>	82.0 ± 17.3 <sup>a</sup>	157.2 ± 31.4 <sup>a</sup>	>0.05	>0.05	<0.05

Data are shown as means ± SEM, n = 7-8. Data with different letters are significantly different, *p* < 0.05. *αSma*, alpha smooth muscle actin; C, control diet; DE, diet effect; DExSoBE, interaction between diet and SoB; FFC, fat-, fructose-, and cholesterol-rich diet; NASH, non-alcoholic steatohepatitis; SoB, sodium butyrate; SoBE; sodium butyrate effect; *Tgfb*, transforming necrosis factor beta.

**Table S3. Effect of SoB on NO<sub>2</sub><sup>-</sup> concentration as well as mRNA expression of *iNos* and proinflammatory mediators in J774A.1 cells.**

	Treatment groups				<i>p</i> (two-way ANOVA)		
	C	LPS	C + SoB	LPS + SoB	LPSExSoBE	SoBE	LPSE
<b>NO<sub>2</sub><sup>-</sup> (% of control)</b>	100.0 ± 0 <sup>b</sup>	6,379 ± 2,131 <sup>a</sup>	76.6 ± 30.4 <sup>b</sup>	4,240 ± 1,419 <sup>a</sup>	>0.05	>0.05	<0.05
<b><i>iNos</i> mRNA expression (% of control)</b>	100.0 ± 0 <sup>b</sup>	8,399 ± 2,284 <sup>a</sup>	232.3 ± 37.5 <sup>c</sup>	11,823 ± 1,790 <sup>a</sup>	>0.05	<0.05	<0.05
<b><i>Tnfa</i> mRNA expression (% of control)</b>	100.0 ± 0 <sup>b</sup>	607.8 ± 148.3 <sup>a</sup>	137.9 ± 16.3 <sup>b</sup>	698.4 ± 223.6 <sup>a</sup>	>0.05	>0.05	<0.05
<b><i>Il1β</i> mRNA expression (% of control)</b>	100.0 ± 0 <sup>b</sup>	116,264 ± 28,490 <sup>a</sup>	151.0 ± 28.1 <sup>b</sup>	315,263 ± 89,146 <sup>a</sup>	>0.05	>0.05	<0.05
<b><i>Il6</i> mRNA expression (% of control)</b>	100.0 ± 0 <sup>b</sup>	716 ± 131 <sup>a</sup>	142.1 ± 20.3 <sup>b</sup>	851.3 ± 210.8 <sup>a</sup>	>0.05	>0.05	<0.05

Data are shown as means ± SEM, n = 5. Data with different letters are significantly different, *p* < 0.05. C, control; Il, interleukin; LPS, lipopolysaccharide; LPSE, LPS effect; LPSExSoBE, interaction between LPS and SoB; *iNos*, inducible nitric oxide synthase; NO, nitric oxide; SoB, sodium butyrate; SoBE; sodium butyrate effect; *Tnfa*, tumor necrosis factor alpha.

**Table S4. Effect of SoB on mRNA expression of melatonin receptor, Aanat and Hiomt in small intestinal tissue of an everted gut sac model.**

	Treatment groups		
	C	3 mM SoB	6 mM SoB
<b><i>Mtr1a</i> mRNA expression</b> (% of control)	100.0 ± 32.9 <sup>a</sup>	134.7 ± 15.0 <sup>a</sup>	178.0 ± 33.5 <sup>a</sup>
<b><i>Mtr1b</i> mRNA expression</b> (% of control)	ND	ND	ND
<b><i>Aanat</i> mRNA expression</b> (% of control)	100.0 ± 15.3 <sup>a</sup>	81.78 ± 11.0 <sup>a</sup>	105 ± 9.7 <sup>a</sup>
<b><i>Hioimt</i> mRNA expression</b> (% of control)	ND	ND	ND

Data are shown as means ± SEM, n = 5-6. Data with different letters are significantly different,  $p < 0.05$ . C, everted gut sacs incubated only in 1x Krebs-Henseleit-bicarbonate-buffer; Aanat, serotonin N-acetyltransferase; Hiomt, hydroxyindole-O-methyltransferase; *Mtr*, melatonin receptor; ND, not detectable; SoB, sodium butyrate.