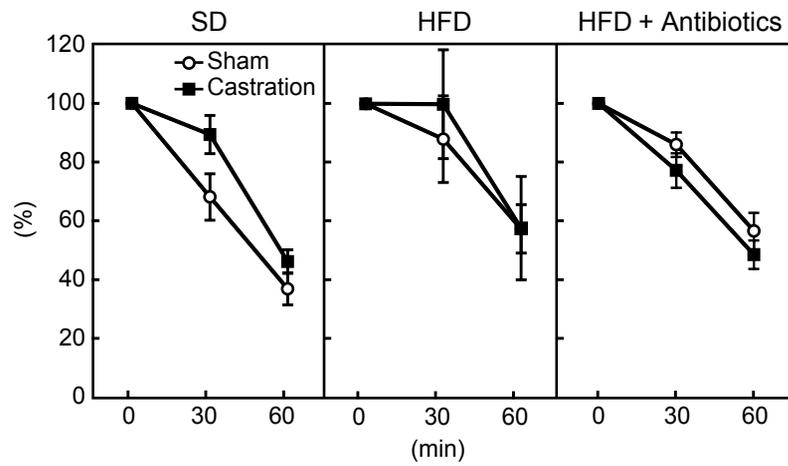


Castration influences intestinal microflora and induces abdominal obesity in high-fat diet-fed mice

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Supplemental Figure S1. Insulin tolerance test (experiments 1–3).

Mice at 22-weeks old were fasted for 6 h and human insulin (1 U/kg) were intraperitoneally injected. Blood glucose levels were measured and expressed as percentage change from baseline (0 min). (n=6, SD sham or castration; n=7, HFD without antibiotics sham or castration; n=9, HFD with antibiotics sham; n=6, HFD with antibiotics castration)

Supplementary Table S1. Relative weight of organs (% of body weight) of 13-week old mice (experiment 4)

	HFD		HFD+Antibiotics	
	Sham	Castration	Sham	Castration
Visceral WAT				
Mesenteric	0.65±0.11 ^b	1.14±0.11 ^a	0.57±0.07 ^b	0.55±0.10 ^b
Perirenal/retroperitoneal	0.54±0.11 ^b	1.07±0.11 ^a	0.51±0.07 ^b	0.45±0.11 ^b
Epididymal	1.49±0.21 ^a	1.56±0.16 ^a	1.18±0.17 ^a	1.09±0.13 ^a
Subcutaneous WAT				
Inguinal	1.33±0.12 ^b	2.23±0.16 ^a	1.23±0.12 ^b	1.26±0.22 ^b
Skeletal muscle				
Quadriceps	1.43±0.05 ^a	1.22±0.06 ^a	1.35±0.08 ^a	1.37±0.04 ^a
Hamstrings	3.03±0.18 ^a	2.86±0.05 ^a	3.00±0.08 ^a	2.78±0.12 ^a

Data are expressed as means ± SEM. Data were analysed by one-way ANOVA followed by Tukey-Kramer's post-hoc test. Different letters mean statistical differences ($p < 0.05$).

Supplementary Table S2. Composition of the diets (experiments 1–4)

	SD	HFD
Ingredients (g/kg)		
Casein	200.00	200.00
L-Cystine	3.00	3.00
Cornstarch	397.44	167.44
α -Cornstarch	132.00	132.00
Sucrose	100.00	100.00
Corn oil	70.00	20.00
Lard	0.00	1400.00
Beef tallow	0.00	1400.00
Cellulose	50.00	50.00
Mineral Mix (AIN-93G)	35.00	35.00
Vitamin Mix (AIN-93)	10.00	10.00
Choline chloride	2.50	2.50
Tert-butylhydroquinone	0.06	0.06
Energy density (kcal/g diet)	3.69	4.82
Macronutrients (% total energy)		
Protein	19%	15%
Carbohydrate	62%	33%
Fat	19%	52%

Supplementary Table S3. Primers for amplification of genes by qRT-PCR

Gene	Sequences of primers	Anneal Temp (°C)	Steps
mouse <i>Acc</i>	5'-GGAGATGTACGCTGACCGAGAA-3' 5'-ACCCGACGCATGGTTTTCA-3'	60	2
mouse <i>Atgl</i>	5'-TGTGGCCTCATTCTCCTAC-3' 5'-TCGTGGATGTTGGTGGAGCT-3'	58	2
mouse <i>Cd36</i>	5'-GCCAAGCTATTGCGACATGA-3' 5'-CAATGGTTGTCTGGATTCTGG-3'	58	2
mouse <i>Cpt1a</i>	5'-GATCTACAATCCCCTCTGCTCT-3' 5'-TAGAGCCAGACCTTGAAGTAACG-3'	60	2
mouse <i>Fasn</i>	5'-TGGGTTCTAGCCAGCAGAGT-3' 5'-ACCACCAGAGACCGTTATGC-3'	60	2
mouse <i>Hsl</i>	5'-GCTGGGCTGTCAAGCACTGT-3' 5'-GTAAGTGGGTAGGCTTGCCAT-3'	58	2
mouse <i>Lpl</i>	5'-GTGGCCGAGAGCGAGAACAT-3' 5'-GCTTTCACCTCGGATCCTCTC-3'	60	2
mouse <i>Mtp</i>	5'-CATCTCCACAGTGCAGTTCTCACA-3' 5'-GGAGTTCACATCCGGCCACTA-3'	58	2
mouse <i>Pparg</i>	5'-GGAAGCCCTTTGGTGACTTTATGG-3' 5'-GCAGCAGGTTTCTTGGATGTC-3'	58	2
mouse <i>Ppara</i>	5'-AATGCAATTCGCTTTGGAAG-3' 5'-GGCCTTGACCTTGTTTCATGT-3'	60	2
mouse <i>Srebp1c</i>	5'-AGCTGTCGGGGTAGCGTCTG-3' 5'-GAGAGTTGGCACCTGGGCTG-3'	55	2
mouse β -Actin	5'-TTGCTGACAGGATGCAGAAG-3' 5'-GTACTTGCGCTCAGGAGGAG-3'	60	2
<i>Bacteroidetes</i> phylum	5'-GGAGYATGTGGTTTAATTCGAAGCA-3' 5'-AGCTGACGACAACCATGCAG-3'	60	3
<i>Firmicutes</i> phylum	5'-GGARCATGTGGTTTAATTCGATGAT-3' 5'-AGCTGACGACAACCATGCAC-3'	60	3
<i>Bifidobacterium</i> spp.	5'-TCGCGTCYGGTGTGAAAG-3' 5'-CCACATCCAGCRTCCAC-3'	60	3
<i>Lactobacillus</i> spp.	5'-AGCAGTAGGGAATCTTCCA-3' 5'-CACCGCTACACATGGAG-3'	60	3
All bacteria	5'-GTGSTGCAYGGYTGTCGTCA-3' 5'-ACGTCRTCCMCACCTTCCTC-3'	60	3