Supplementary File

Title Maternal low-dose aspartame and stevia consumption with an obesogenic diet alters metabolism, gut microbiota, and mesolimbic reward system in rat dams and their offspring.

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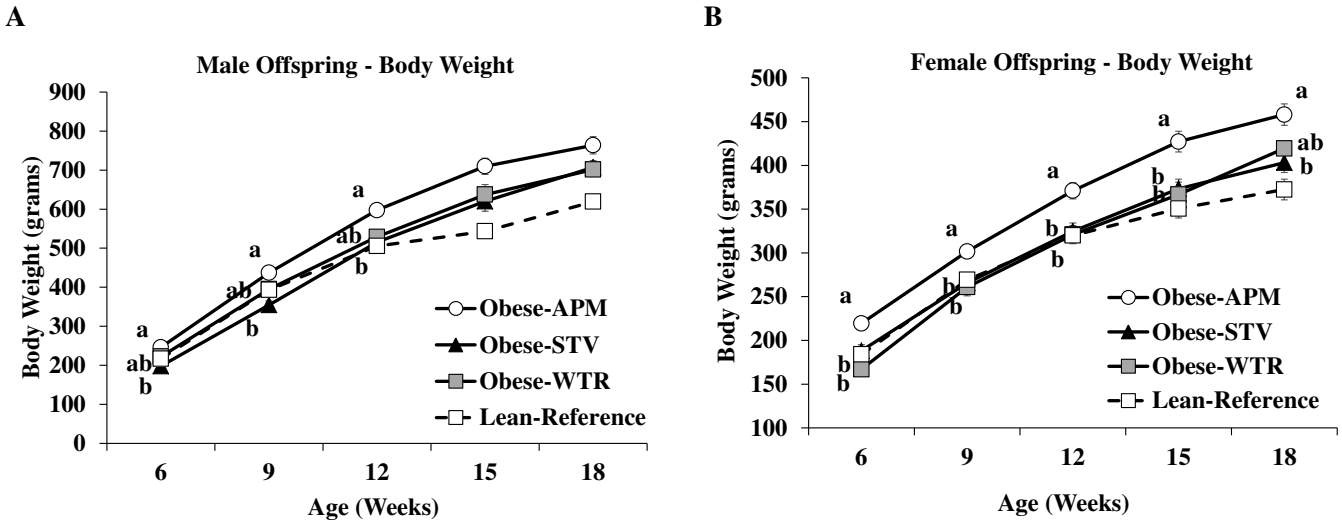
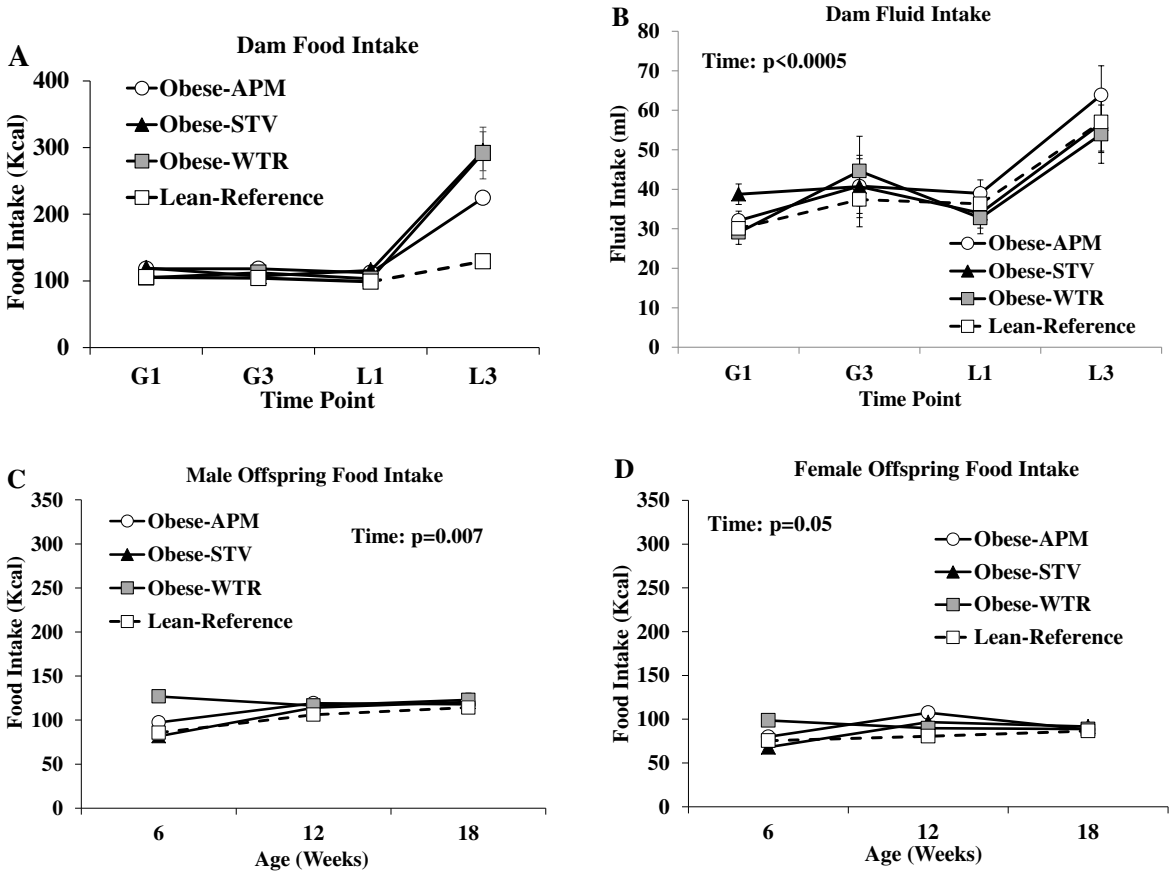
Supplementary figure 1



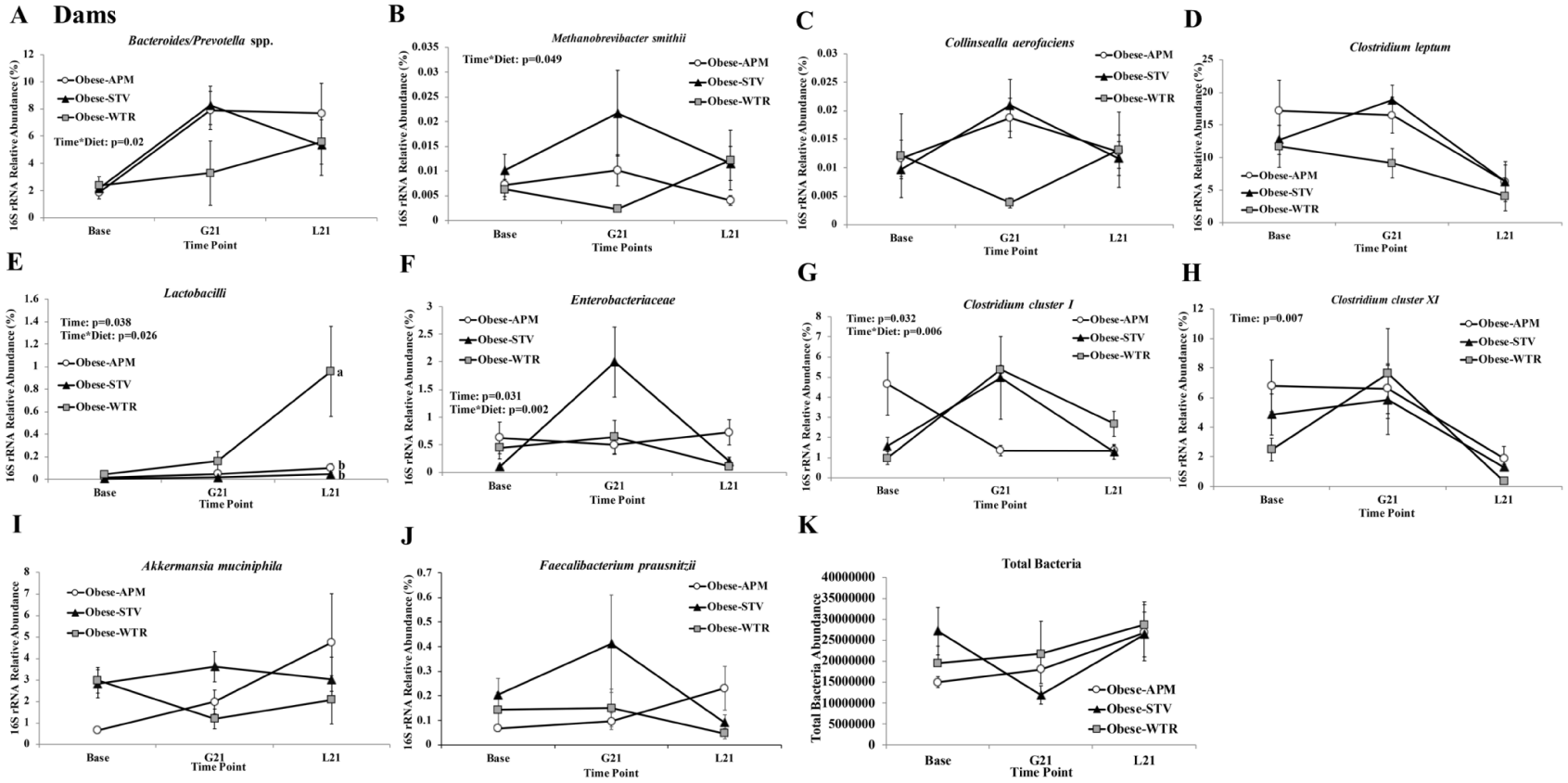
Supplementary figure 1 Maternal aspartame consumption increased body weight in male and female offspring. (A) Male offspring body weight; (B) Female offspring body weight. Values are mean ± SEM. Obese-APM, obese aspartame (n=9M, 8F); Obese-STV, obese stevia (n=7M & F); Obese-WTR, obese control (n=6M & F); Lean-Reference, lean control (n=10M & F, not included in statistical analysis). ^{a,b} superscripts indicate significant differences between groups of the same sex where labeled means without a common superscript letter differ, $p \leq 0.05$ (i.e. 'a' and 'b' differ; 'ab' does not differ from 'a' or 'b').

Supplementary figure 2



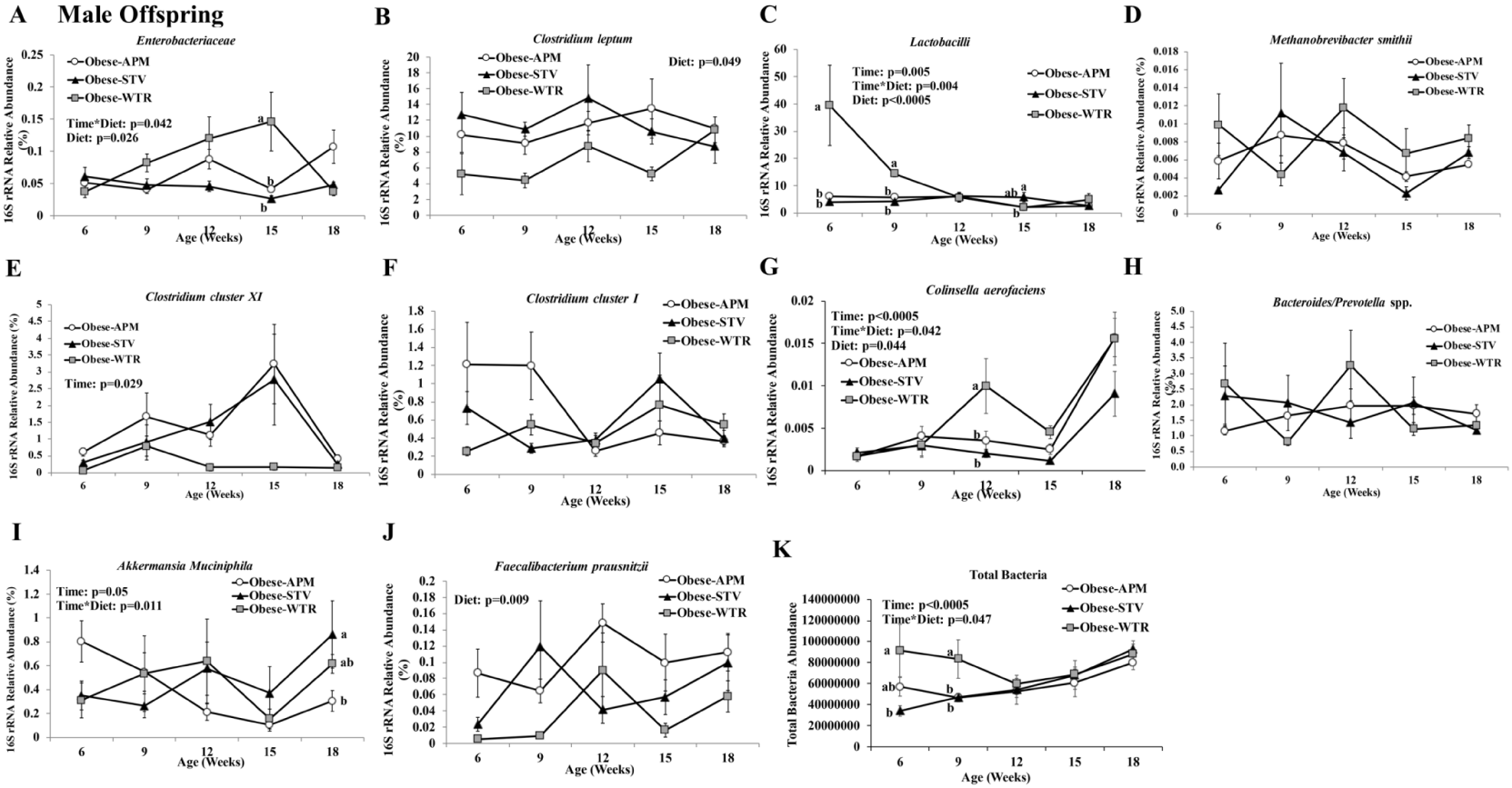
Supplementary figure 2 Average daily food & fluid intake of dams during gestation/lactation and food intake of offspring. (A) Dam food intake; (B) Dam fluid intake; (C) Male offspring food intake; (D) Female offspring food intake. Values are mean ± SEM. Obese-APM, obese aspartame (n=10 dams gestation/n=9 lactation, n=9M and 8F offspring); Obese-STV, obese stevia (n=8 dams gestation/n=7 lactation, n=7 M & F offspring); Obese-WTR, obese control (n=13 dams gestation/n=6 lactation, n=6 M & F offspring); Lean-Reference, lean control (n=14 dams gestation/n=11 lactation, n=10 M & F offspring). No diet differences found.

Supplementary figure 3



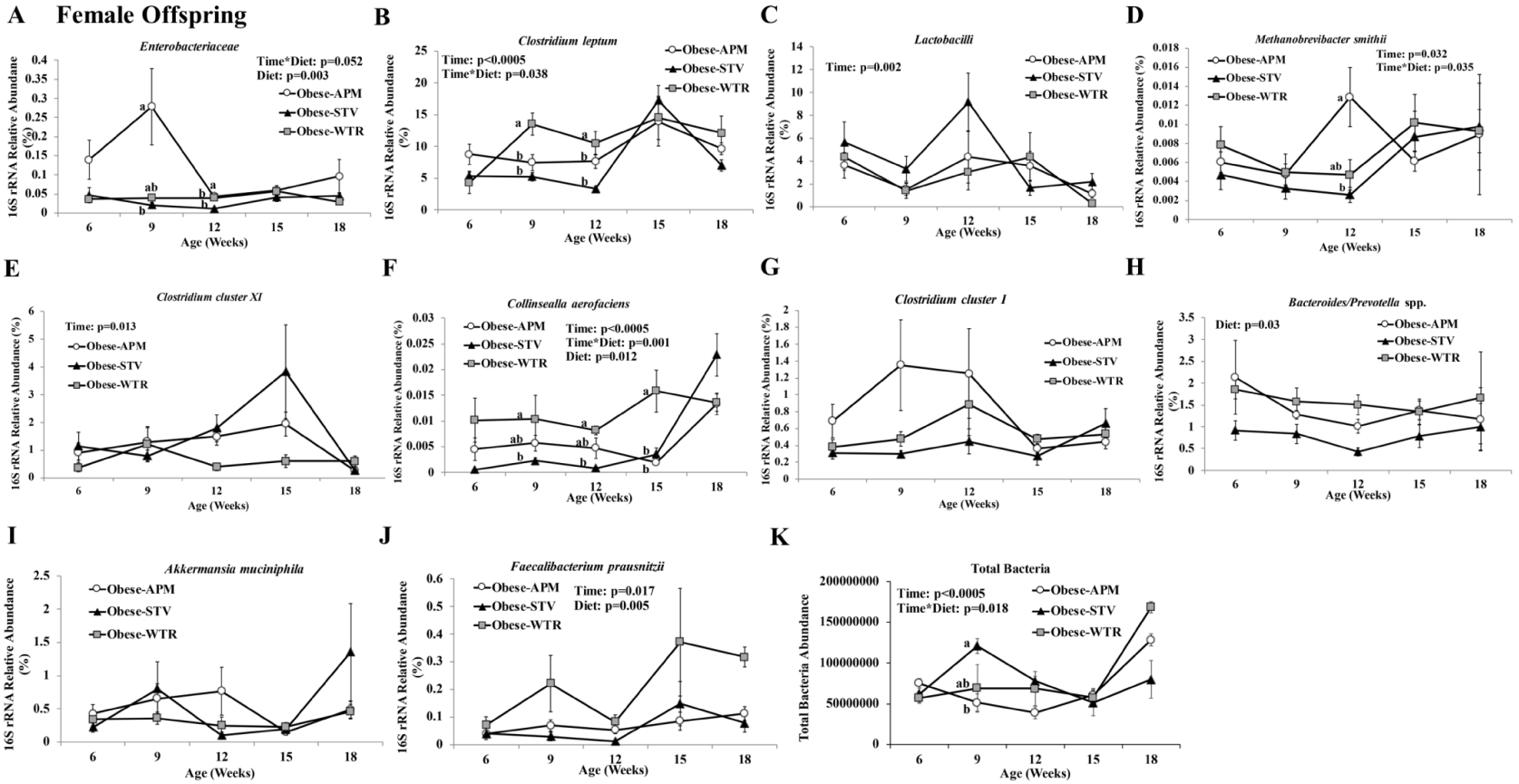
Supplementary figure 3 Dams longitudinal fecal microbiota alterations resulting from maternal LCS and HFS consumption measured with qPCR. Relative abundance: (A) *Bacteroides/Prevotella* spp.; (B) *Methanobrevibacter smithii*; (C) *Collinsella aerofaciens*; (D) *Clostridium leptum*; (E) *Lactobacilli*; (F) *Enterobacteriaceae*; (G) *Clostridium* cluster I; (H) *Clostridium* cluster XI; (I) *Akkermansia muciniphila*; (J) *Faecalibacterium prausnitzii*; (K) Total bacteria. Values are mean ± SEM. Obese-APM, obese aspartame (n=9); Obese-STV, obese stevia (n=7); Obese-WTR, obese control (n=6). ^{a,b} superscripts indicate significant differences between groups where labeled means without a common superscript letter differ, $p \leq 0.05$.

Supplementary figure 4



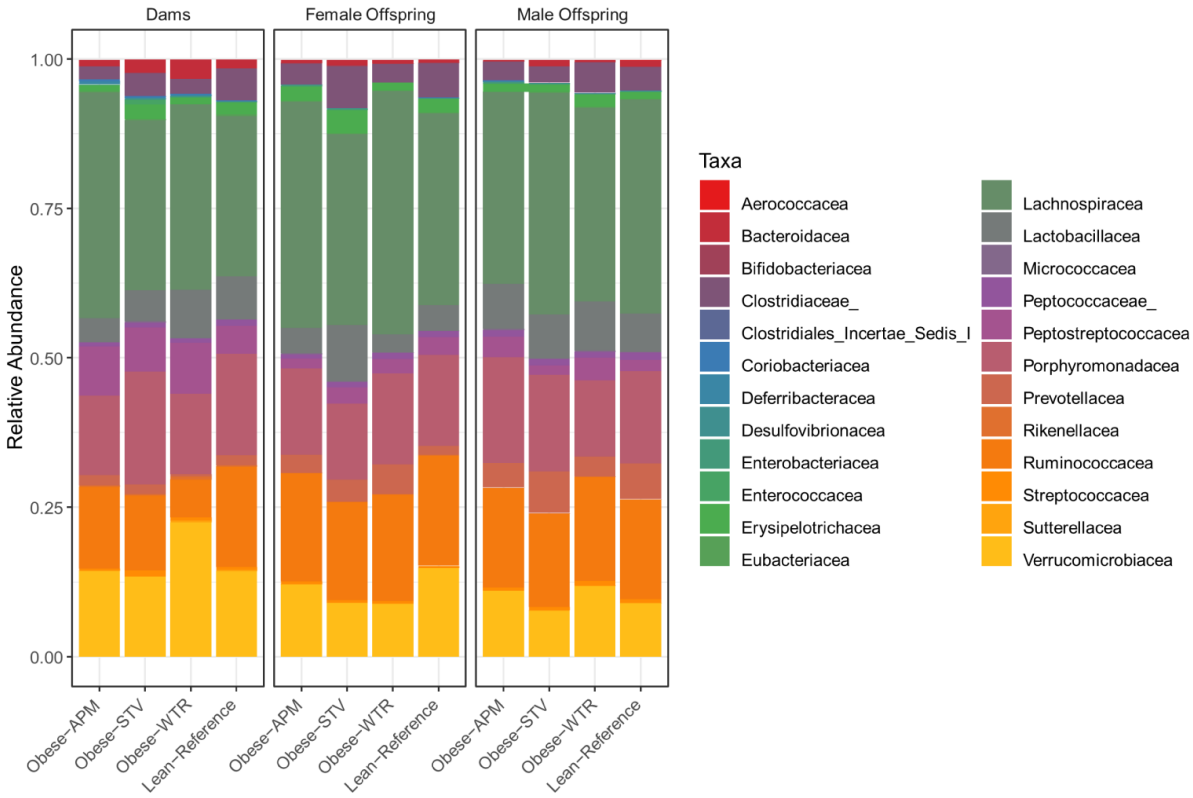
Supplementary figure 4 Male offspring longitudinal fecal microbiota alterations resulting from maternal LCS consumption measured with qPCR. Relative abundance of: (A) *Enterobacteriaceae*; (B) *Clostridium leptum*; (C) *Lactobacilli*; (D) *Methanobrevibacter smithii*; (E) *Clostridium* cluster XI; (F) *Clostridium* cluster I; (G) *Collinsella aerofaciens*; (H) *Bacteroides/Prevotella* spp.; (I) *Akkermansia muciniphila*; (J) *Faecalibacterium prausnitzii*; (K) Total Bacteria. Values are mean ± SEM. [a,b] superscripts indicate significant differences between groups where labeled means without a common superscript letter differ, $p \leq 0.05$. Obese-APM, obese aspartame (n=9); Obese-STV, obese stevia (n=7); Obese-WTR, obese control (n=6).

Supplementary figure 5



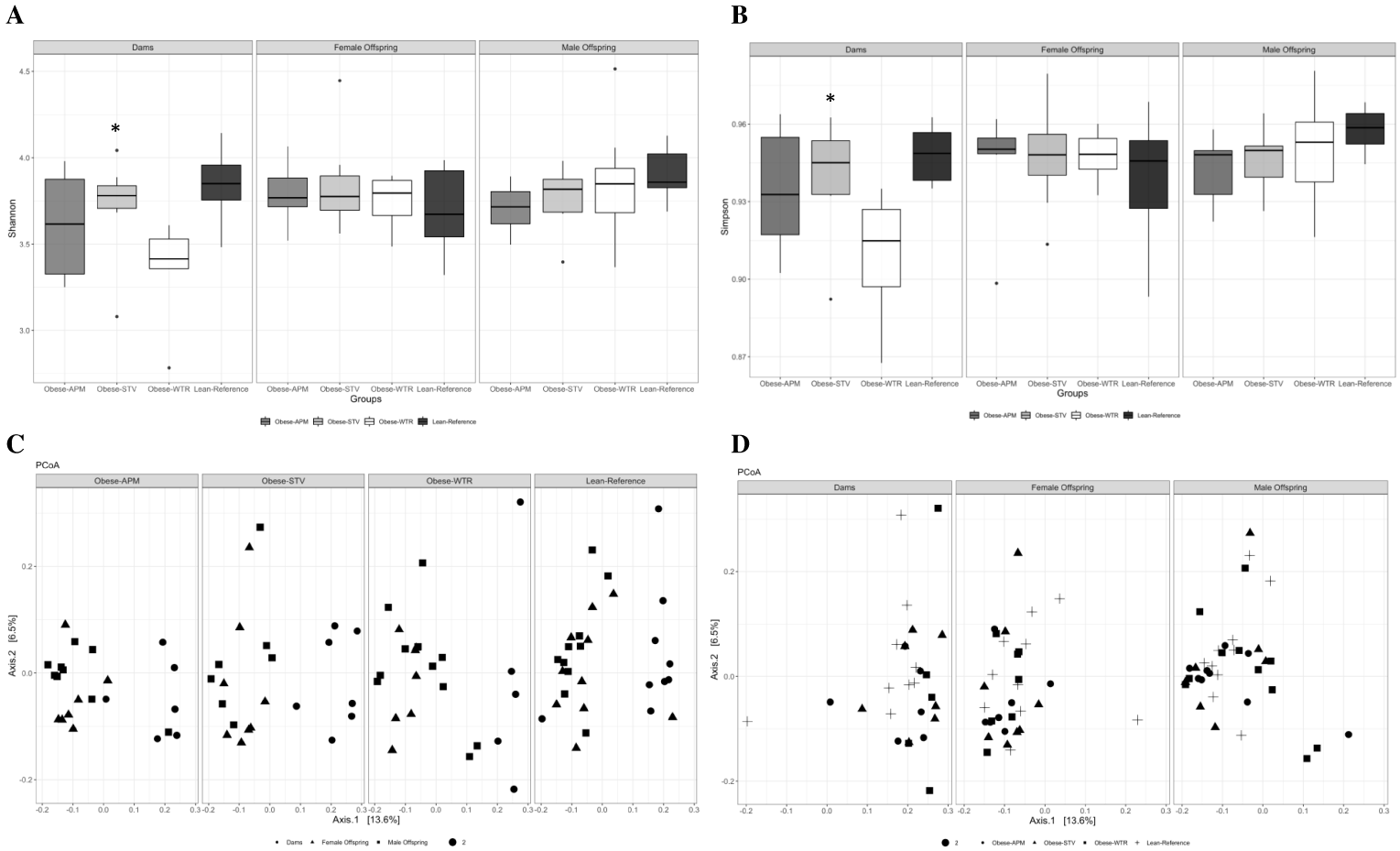
Supplementary figure 5 Female offspring longitudinal fecal microbiota alterations resulting from maternal LCS consumption measured with qPCR. Relative abundance of: (A) *Enterobacteriaceae*; (B) *Clostridium leptum*; (C) *Lactobacilli*; (D) *Methanobrevibacter smithii*; (E) *Clostridium* cluster XI; (F) *Collinsella aerofaciens*; (G) *Clostridium* cluster I; (H) *Bacteroides/Prevotella* spp.; (I) *Akkermansia muciniphila*; (J) *Faecalibacterium prausnitzii*; (K) Total Bacteria. Values are mean ± SEM. [a,b] superscripts indicate significant differences between groups where labeled means without a common superscript letter differ, p≤0.05. Obese-APM, obese aspartame (n=8); Obese-STV, obese stevia (n=7); Obese-WTR, obese control (n=6).

Supplementary figure 6



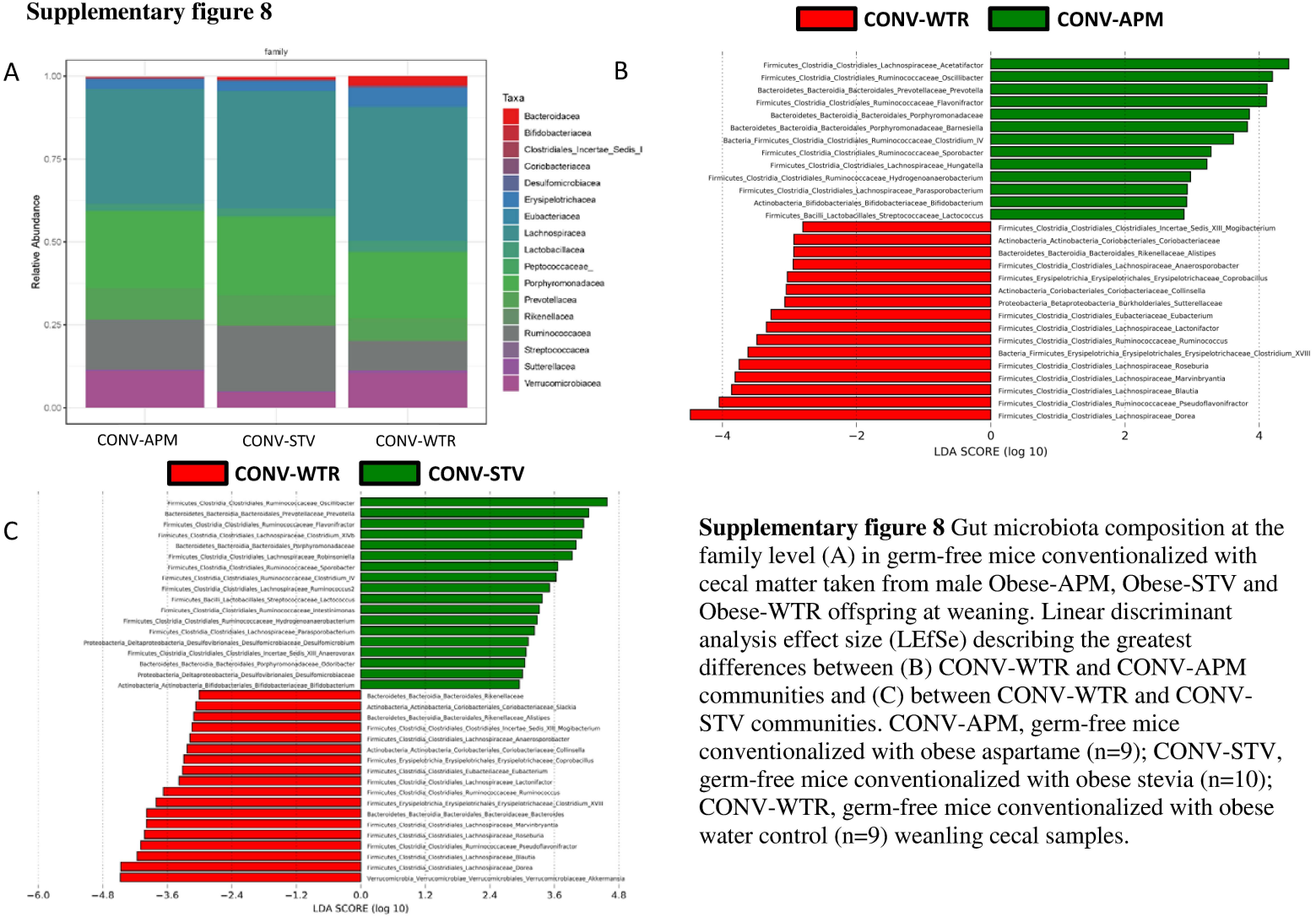
Supplementary figure 6 Cecal microbiota of dams (consuming aspartame or stevia alongside an obesogenic diet) at weaning and offspring at 18 weeks of age. Stacked bars show relative abundance of cecal microbiota at the family level. Obese-APM, obese aspartame (n=7 dams, n=9M and 8F offspring); Obese-STV, obese stevia (n=7 dams, n=7 M & F offspring); Obese-WTR, obese control (n=5 dams, n=5 M & F offspring); Lean-Reference, lean control (n=10 dams, n=10 M & F offspring).

Supplementary figure 7



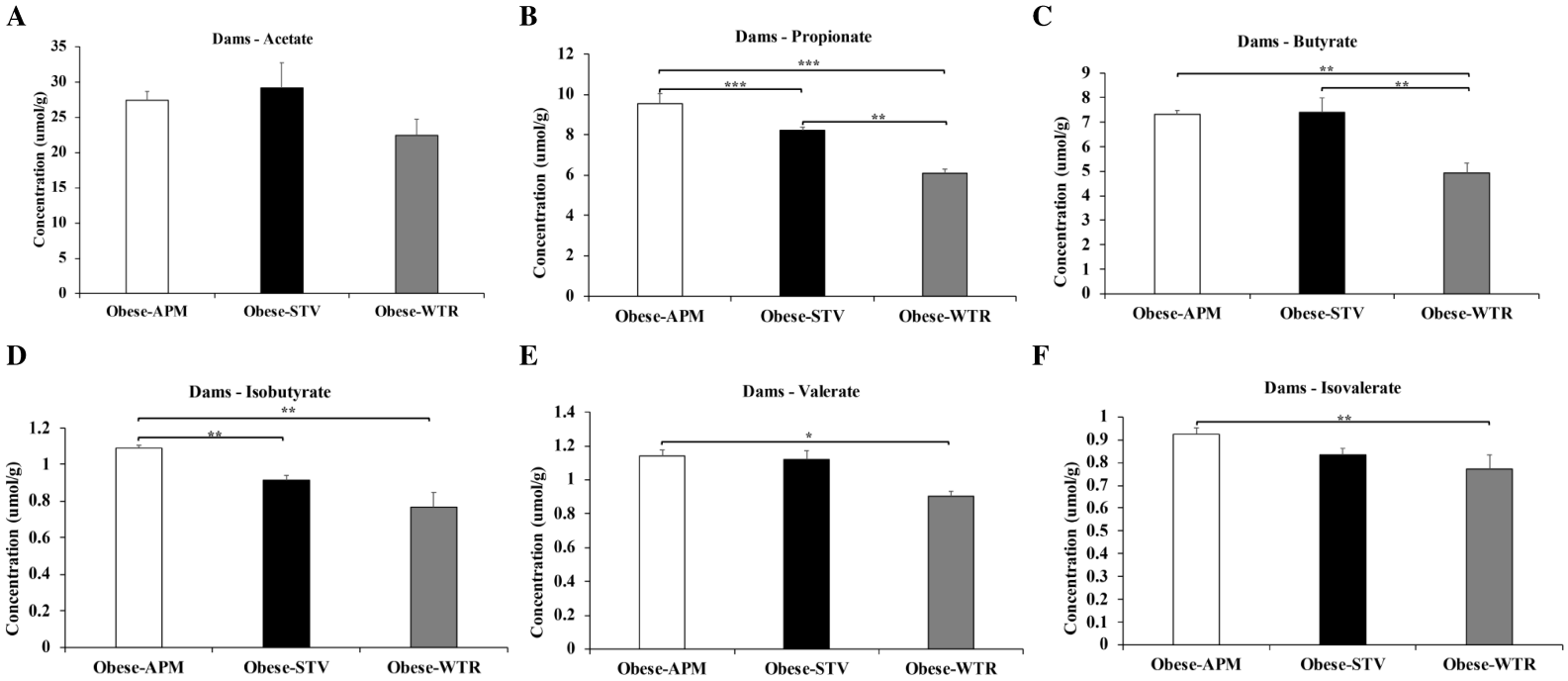
Supplementary figure 7 Alpha diversity as (A) Shannon and (B) Simpson index and beta diversity according to (C) treatment and (D) generation. Alpha diversity did not differ with the exception of higher diversity in Obese-STV than Obese-WTR dams (*p<0.05). There were trends towards differences in beta diversity in dams (p=0.06) and their offspring (males p=0.17; females p=0.08). Obese-APM, obese aspartame (n=7 dams, n=9M and 8F offspring); Obese-STV, obese stevia (n=7 dams, n=7 M & F offspring); Obese-WTR, obese control (n=5 dams, n=5 M & F offspring); Lean-Reference, lean control (n=10 dams, n=10 M & F offspring).

Supplementary figure 8



Supplementary figure 8 Gut microbiota composition at the family level (A) in germ-free mice conventionalized with cecal matter taken from male Obese-APM, Obese-STV and Obese-WTR offspring at weaning. Linear discriminant analysis effect size (LEfSe) describing the greatest differences between (B) CONV-WTR and CONV-APM communities and (C) between CONV-WTR and CONV-STV communities. CONV-APM, germ-free mice conventionalized with obese aspartame (n=9); CONV-STV, germ-free mice conventionalized with obese stevia (n=10); CONV-WTR, germ-free mice conventionalized with obese water control (n=9) weanling cecal samples.

Supplementary figure 9



Supplementary figure 9 Cecal SCFA concentration in dams. Dams cecal (A) acetate; (B) propionate; (C) butyrate; (D) isobutyrate; (E) valerate; (F) isovalerate concentration. Values are mean ± SEM. * indicates $p<0.05$; ** indicates $p<0.01$; *** indicates $p<0.0005$. Obese-APM, obese aspartame (n=9); Obese-STV, obese stevia (n=7); Obese-WTR, obese control (n=6).

Supplementary table 1 Experimental group litter information.

	Obese-APM	Obese-STV	Obese-WTR	Lean-Reference
Pregnancy:				
Obese females allocated to groups, *n*	15	15	15	15
Pregnancies carried to completion, *n*	10	8	13	14
Unsuccessful pregnancies, *n*	5	7	2	1
Total litters carried forward from birth, *n*	**10**	**8**	**13**	**14**
Lactation:				
Litters lost to cannibalism, *n*	1	1	6	0
Litters lost to pup death, *n*	0	0	1	3
Total litters carried forward from weaning, *n*	**9**	**7**	**6**	**11**
Excluded from testing due to sufficient *n*	**-**	**-**	**-**	1

Obese-APM, obese aspartame; Obese-STV, obese stevia; Obese-WTR, obese water control; Lean-Reference, lean control.