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Supplemental Information

The Role of Elevated Branched-Chain Amino Acids in the Effects of Vertical Sleeve Gastrectomy to Reduce Weight and Improve Glucose Regulation

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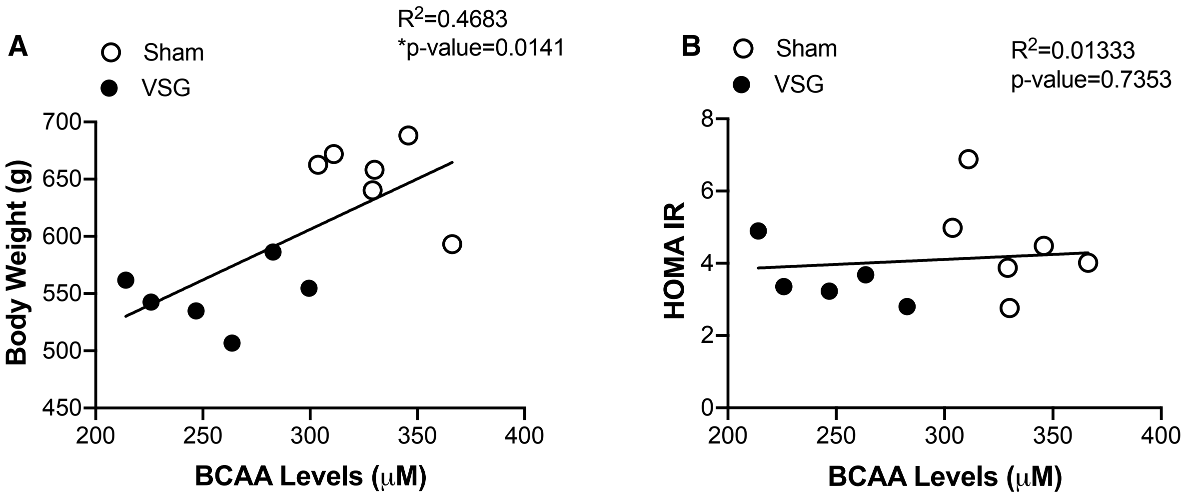

Figure S1. Circulating BCAA levels correlate with body weight, but not insulin resistance in Sham and VSG rats fed HFD. Related to Figure 1. Circulating BCAA levels (14 days post-surgery) are positively correlated with **A)** body weight change (28 days post-surgery), but not **B)** HOMA IR (28 days post-surgery). Animal number n=5-6/group. Data analyzed by simple linear regression analysis * $p<0.05$.

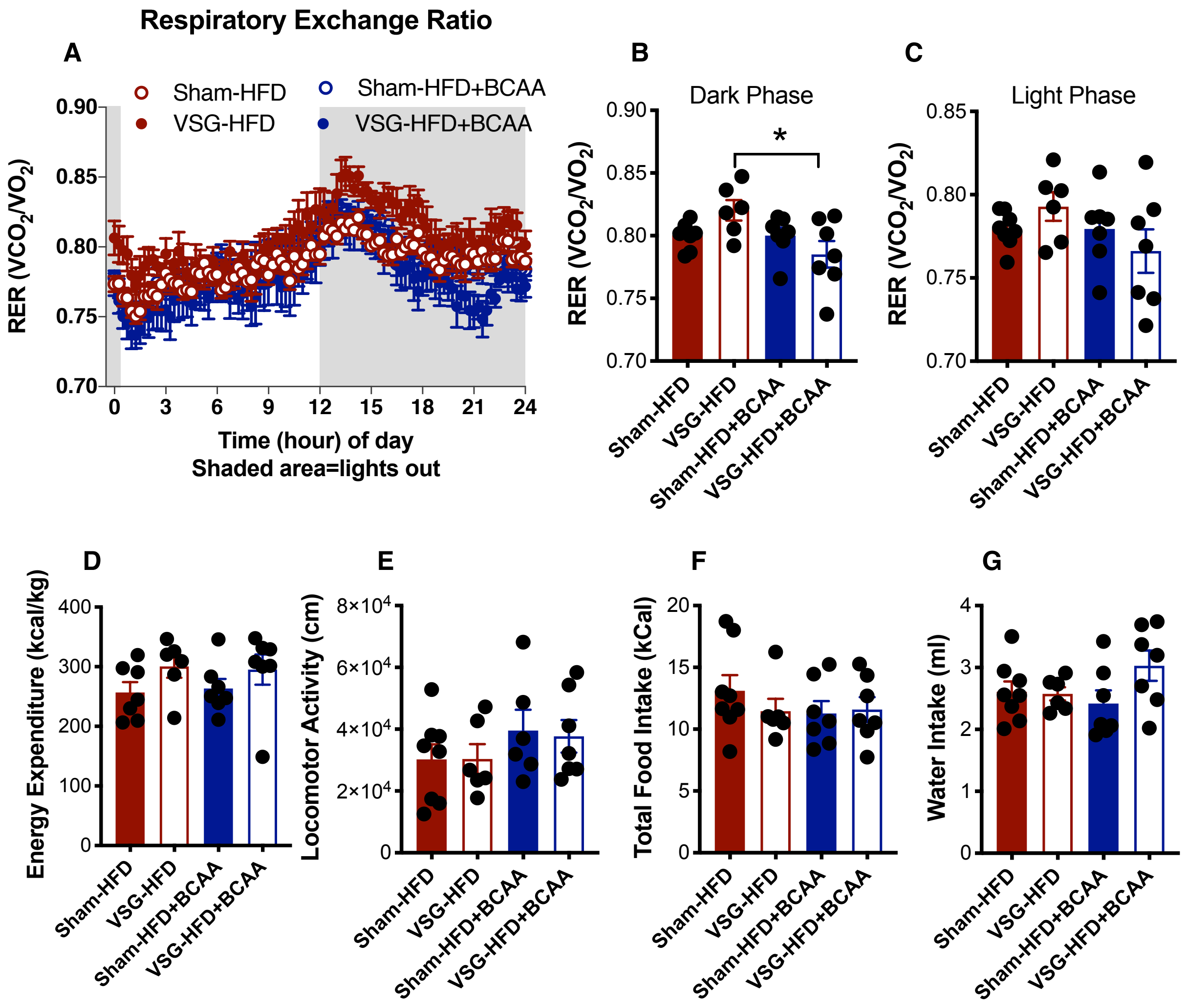

Figure S2. HFD supplementation with BCAA lowers Respiratory Exchange Ratio after VSG. Related to Figures 2 and 3. A) Respiratory Exchange Ratio RER (VCO2/VO2) for 24-hour period. Respiratory Exchange Ratio RER (VCO2/VO2) for **B)** Dark and **C)** Light phase. **D)** Energy Expenditure (kCal/kg). **E)** Locomotor Activity (cm). **F)** Total Food intake (kCal) and **G)** Total Water Intake for Sham-HFD (n=8), VSG-HFD (n=6), Sham-HFD+BCAA (n=7) and VSG-HFD+BCAA (n=7). Data represent average values for three consecutive days of recording. Shaded region in panel A represents dark phase. Data are shown as means ± S.E.M. * $p<0.05$; (2-Way ANOVA with Tukey's post-test).

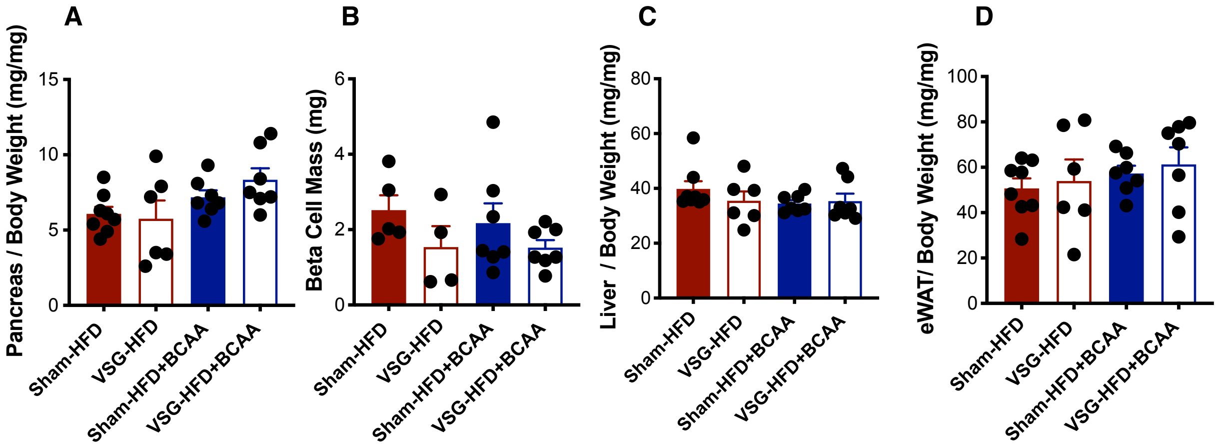

Figure S3. Dietary supplementation of high fat diet with BCAA does not alter pancreas weight, pancreatic beta cell mass, liver and epididymal white adipose tissue (eWAT). Related to Figures 2 and 3. A) Pancreas weights normalized to body weight in Sham HFD (n=8), VSG-HFD (n=6), Sham-HFD+BCAA (n=7) and VSG-HFD+BCAA (n=7). **B)** Pancreatic beta cell mass in Sham HFD (n=5), VSG-HFD (n=4), Sham-HFD+BCAA (n=7) and VSG-HFD+BCAA (n=7). **C)** Liver weights normalized to body weight in Sham HFD (n=8), VSG-HFD (n=6), Sham-HFD+BCAA (n=7) and VSG-HFD+BCAA (n=7). **D)** Epididymal white adipose tissue (eWAT) weight normalized to body weight in Sham HFD (n=5-8), VSG-HFD (n=4-6), Sham-HFD+BCAA (n=7) and VSG-HFD+BCAA (n=7). Data are shown as means ± S.E.M. * $p<0.05$; (2-Way ANOVA with Tukey's post-test).

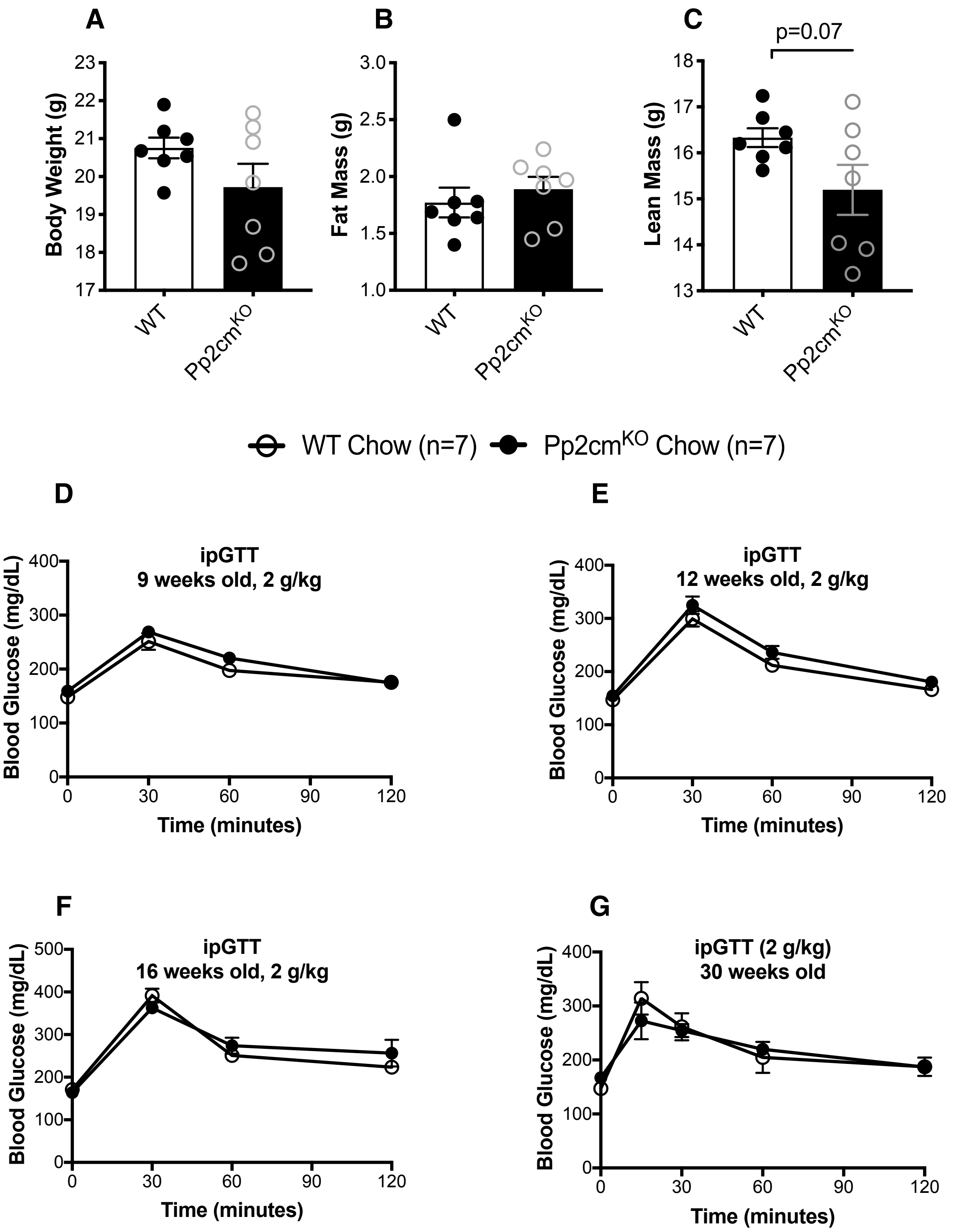

Figure S4. Impaired BCAA catabolism does not lead to glucose intolerance in standard chow-fed mice. Related to Figures 6 and 7. A) Body weight, **B)** Fat mass and **C)** Lean mass of 6-week old Pp2cm^{KO} and littermate WT controls fed standard chow diet. Intraperitoneal tolerance tests (ipGTT; 2 g/kg) were performed in D) 9-weeks old, E) 12-weeks old, F) 16-weeks old and G) 30-weeks old standard chow-fed Pp2cm^{KO} and littermate WT controls (n=7/genotype). Data are shown as means ± S.E.M. * $p<0.05$; (Student's 2-tailed *t* test).

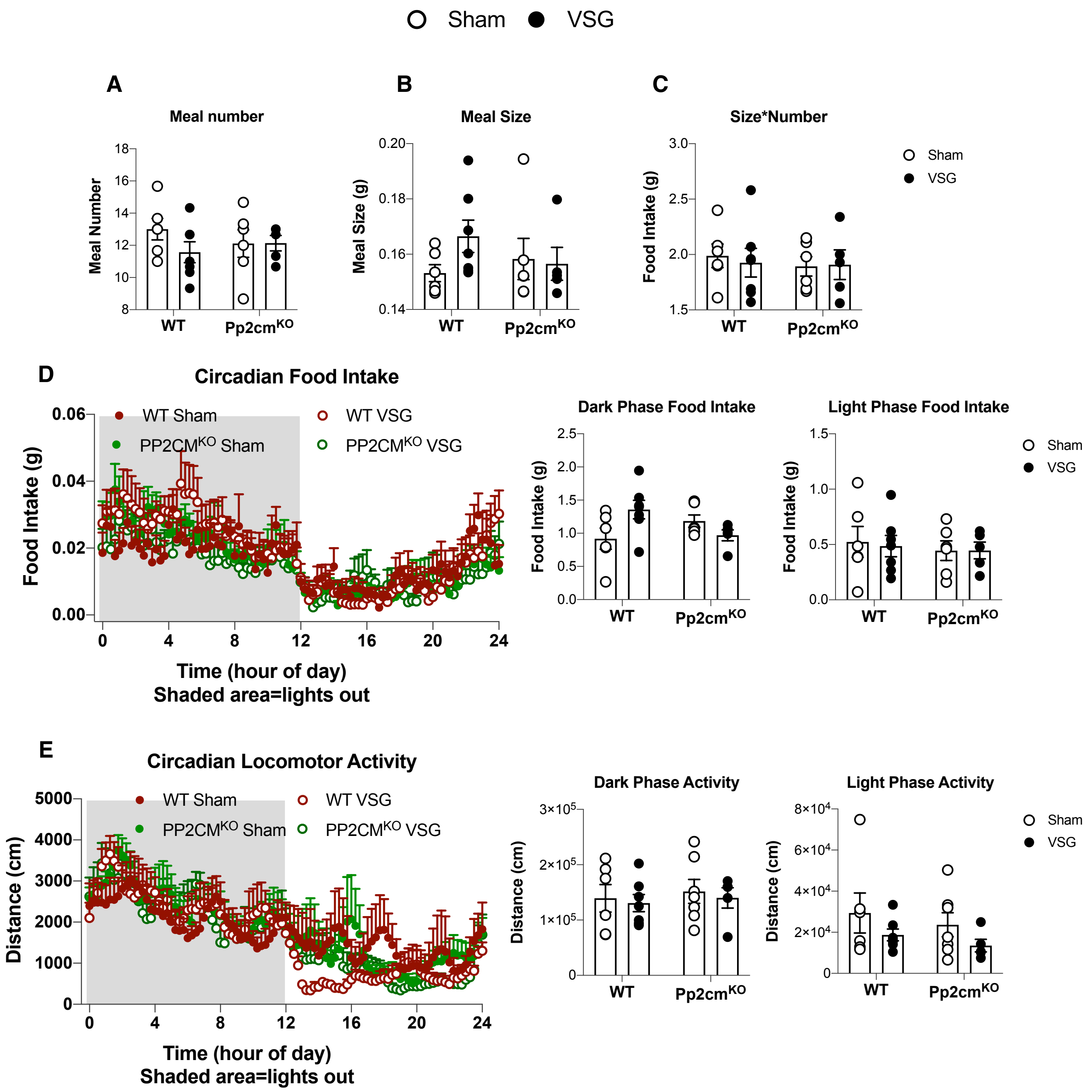

Figure S5. Impaired BCAA catabolism does not change food intake and locomotor activity in Sham and VSG mice. Related to Figures 6 and 7. A) Meal size, **B)** Meal number, and **C)** Meal size*Number. **D)** Circadian, **E)** Dark phase, and **F)** Light phase food intake. **G)** Circadian, **H)** Dark phase, and **I)** Light phase locomotor activity. Animals: WT HFD-Sham (n=7), WT HFD-VSG (n=6), Pp2cm^{KO} HFD-Sham (n=5), Pp2cm^{KO} HFD-VSG (n=5). Data are shown as means ± S.E.M. * $p<0.05$; (2-Way ANOVA with Tukey's post-test).

Table S1. Special diet composition formulated and purchased by Research Diets, Inc. (New Jersey, US). Related to Figures 2 and 3.

Product	**Control HFD (gm) D16121101**	**HFD+BCAA (gm) D16121102**
Casein, 80 Mesh	100	100
L-Cystine	3.35	1.05
L-Isoleucine	4.2	16.8
L-Leucine	7.74	30.96
L-Lysine	6.5	2.04
L-Methionine	2.3	0.72
L-Phenylalanine	4.4	1.38
L-Threonine	3.35	1.05
L-Tryptophan	1.05	0.33
L-Valine	5	20
L-Histidine	2.3	0.72
L-Alanine	2.3	0.72
L-Arginine	3.15	1.0
L-Aspartic Acid	5.7	1.79
L-Glutamic Acid	18.4	5.78
Glycine	1.6	0.50
L-Proline	10.3	3.24
L-Serine	4.75	1.49
L-Tyrosine	4.65	1.46
BCAA (including casein)	16.94	67.76
Non-BCAAs (including casein)	74.10	23.27
Total L-Amino Acids	91.04	91.03
Maltodextrin	125	125
Sucrose	68.8	68.8
Cellulose, BW200	50	50
Corn Starch	0	0
Soybean Oil	25	25
Lard	245	245
Fiber	50	50
Mineral Mix, S10026	10	10
DiCalcium Phosphate	13	13
Calcium Carbonate	5.5	5.5
Potassium Citrate, 1 H2O	16.5	16.5
Sodium Bicarbonate	3.5	3.5
Vitamin Mix, V10001	10	10
Choline Bitartrate	2	2
FD&C Yellow Dye #5	0.05	0.025
FD&C Red Dye #40	0	0.025
Total grams of diet	765.39	765.38
Protein kCal%	18	18
Carbohydrate kCal%	21	21
Fat kCal%	61	61

Table S2. Special diet composition formulated and purchased by Research Diets, Inc. (New Jersey, US). Related to Figures 2 and 3.

Amino Acid	**Control HFD (gm/kg diet) D16121101**	**HFD+BCAA (gm/kg diet) D16121102**	**NRC Requirement (gm/kg diet)**
L-Cystine	5.2	2.2	
L-Isoleucine	10.4	26.9	4
L-Leucine	20.5	50.8	7
L-Lysine	17.1	11.3	4
L-Methionine	6.3	4.3	5*
L-Phenylalanine	11.3	7.3	7.6**
L-Threonine	9.1	6.1	4
L-Tryptophan	2.8	1.8	1
L-Valine	12.6	32.2	5
L-Histidine	6.0	3.9	2
L-Alanine	6.3	4.3	
L-Arginine	8.0	5.2	3
L-Aspartic Acid	15.4	10.3	
L-Glutamic Acid	49.0	32.5	
Glycine	4.0	2.6	
L-Proline	25.1	15.8	
L-Serine	12.8	8.5	
L-Tyrosine	12.1	7.9	
*Per NRC Guidelines, Cystine may replace 50-66.6% of the Methionine requirement	**Per NRC Guidelines, Tyrosine may replace 50% of the Phenylalanine requirement	Reference: Council, N.R. (1995). Nutrient Requirements of Laboratory Animals: Fourth Revised Edition. (Washington (DC)).	