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# Supplementary Materials for

## Improved Rodent Maternal Metabolism But Reduced Intrauterine Growth After Vertical Sleeve Gastrectomy

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Published 21 August 2013, *Sci. Transl. Med.* **5**, 199ra112 (2013)
DOI: 10.1126/scitranslmed.3006505

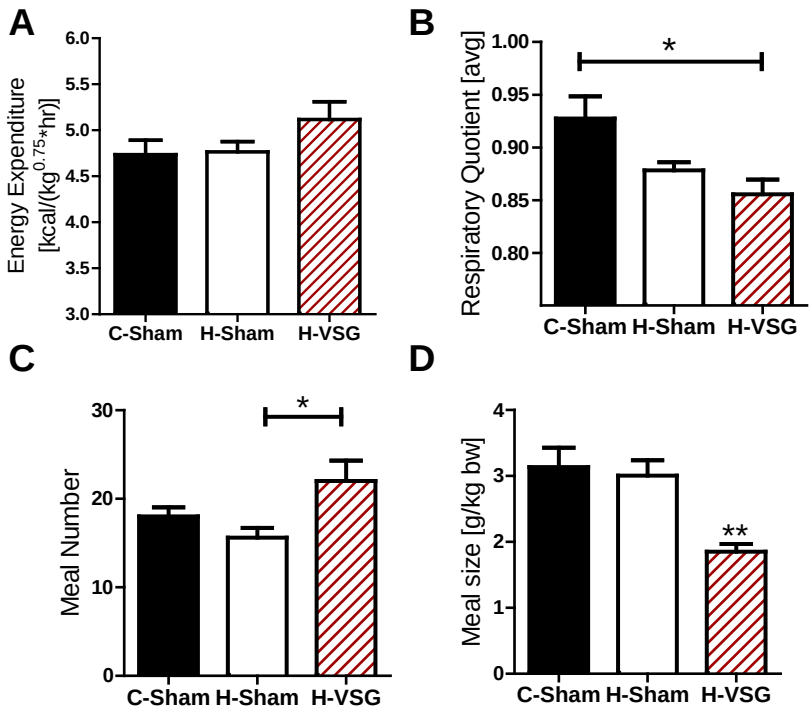
**The PDF file includes:**

**Supplementary Materials and Methods**

**TSE metabolic analysis:** Female rats (post-operative days 100-104) were housed in LabMaster chambers (TSE) Systems GmbH, Bad Homburg, Germany) which allows simultaneous measurement of metabolic performance, home cage activity, and feeding behavior as previously described (*52*).

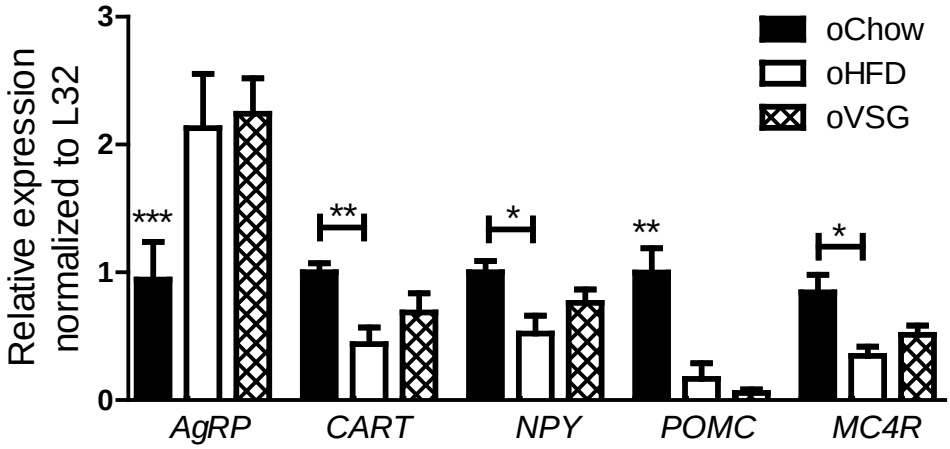
**Semi-quantitative PCR:** Postnatal day 22 rat pups were sacrificed via conscious decapitation, and brains were rapidly removed, frozen and stored at -80° C until processing. Dorsal hippocampus and hypothalamus were hand dissected with aid of a brain block. Total RNA from microdissected tissue was isolated by Trizol reagent (Invitrogen, Carlsbad, CA) and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA), and on column DNAse treatment according to manufacturer's instructions. cDNA was then retrotranscribed from 1-2 μg of total RNA using the SuperScript III First Strand Synthesis Kit (Invitrogen, Carlsbad, CA). The cDNA was diluted and 100 ng of template cDNA from each sample was used to measure mRNA expression of selected genes by real time quantitative PCR using pre-designed and validated Taqman PCR primer/probes sets (Applied Biosystems) on an ABI 7900HT Real-Time PCR System (Applied Biosystems, Inc.). We calculated mRNA expression relative to housekeeping genes, L32 (ABI) using the ΔΔ CT method. The following primer/probes were used: *AGRP* (agouti-related peptide, Rn01431703_g1), *POMC* (pro-opiomelanocortin, Rn00595020_m1), *NPY* (neuropeptide Y, Rn01410145_m1), *CART* (cocaine and amphetamine related transcript, Rn00567382_m1) and *MC4R* (melanocortin 4 receptor, Rn01491866_s1).

## *Supplementary Figure 1*



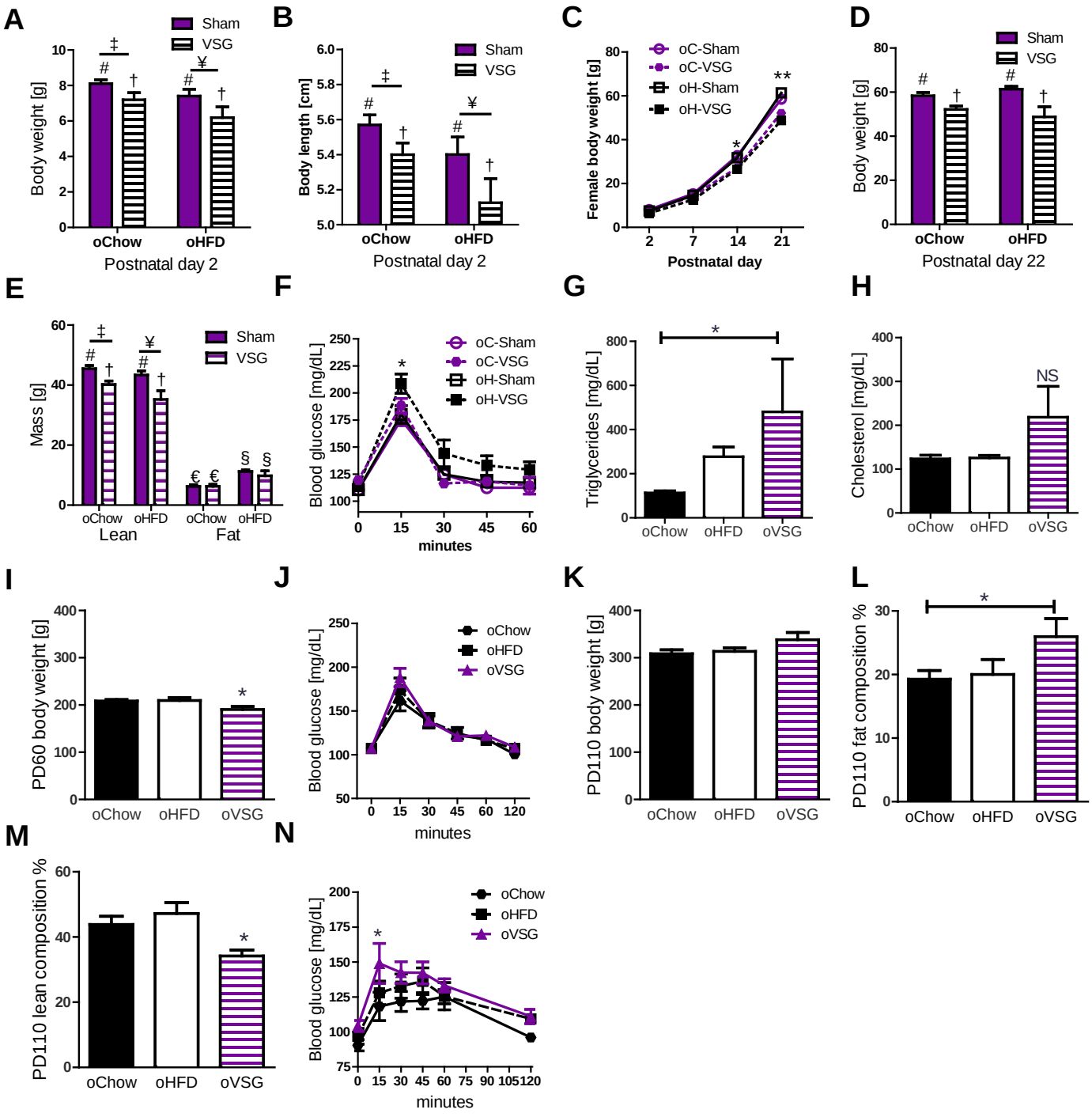

**Supplemental Figure 1**: Metabolic cage analysis of females (post-operative days 100-104). Animals were placed in metabolic chambers for 5 days. **(A)** No differences in energy expenditure were observed. **(B)** VSG-operated females has reduced respiratory quotient with respect to chow-fed controls. (**P*< 0.05) by 1-way ANOVA, with Tukey's *post hoc*, C-Sham vs. H-VSG, (*N*= 6/group). **(C)** Meals were also analyzed. Animals had increased meal number (**P*< 0.05) by 1-way ANOVA, with Tukey's *post hoc*, H-Sham vs. H-VSG, (*N*= 6/group) and **(D)** VSG females also had reduced meal size (**P*< 0.05) by 1-way ANOVA, with Tukey's *post hoc*, (*N*= 6/group) Data are presented as mean SEM.

## *Supplementary Figure 2*




**Supplementary Figure 2. Reverse transcription polymerase chain reaction analysis of hypothalamic brain blocks of postnatal day 22 pups.** *AgRP* (***$P < 0.001$), CART (**$P<0.01$), *NPY* (*$P < 0.05$), *POMC* (**$P < 0.01$)and *MC4R* (*$P < 0.05$) by 1-way ANOVA, with Tukey's *post hoc*, ($N$= 4-7/group). Data are presented as mean SEM.

## *Supplementary Figure 3*



**Supplementary Figure 3. Metabolic characteristics of female rodent offspring. (A)** Average body weight of postnatal day 2 female offspring. Offspring of VSG weighed less in comparison to sham controls. Effect of maternal surgery ($P < 0.05$, # vs. †) and diet ($P < 0.05$, ‡ vs. ¥ ) by 2-way ANOVA, ($N = 3$-10/group). **(B)** Body length of postnatal day 2 VSG offspring was reduced in comparison to sham controls. Effect of maternal surgery ($P < 0.05$, # vs. †) and diet ($P < 0.05$, ‡ vs. ¥ ) by 2-way ANOVA, ($N = 3$-10/group). **(C)** Body weight curve of offspring of chow and HFD-fed, sham and VSG dams throughout postnatal life. Offspring of VSG-treated dams weighed less than offspring of sham-operated dams. Effect of maternal surgery (**$P < 0.01$) and time (***$P < 0.001$) by 2-way repeated-measures ANOVA, ($N = 3$-10/group). **(D)** Body weights at postnatal day 22 of female offspring of VSG were reduced in comparison to offspring of sham-operated dams. Effect of maternal surgery ($P < 0.001$, ‡ vs. ¥ ) by 2-way ANOVA, ($N = 3$-10/group). **(E)** Lean mass measured by NMR of postnatal day 22 male offspring. Effect of maternal surgery ($P < 0.001$, # vs. †) and maternal diet ($P < 0.05$, ‡ vs. ¥) by 2-way ANOVA ($N = 3$-10). Fat mass was also measured by NMR. Effect of maternal diet ($P < 0.001$, € vs.  ) by 2-way ANOVA ($N = 3$-10). **(F)** An intraperitoneal glucose tolerance test dosed at 1.25 g dextrose /kg body weight was performed at weaning. Animals were fasted for 4 hours prior to the test. (*$P < 0.05$) at 15 minute time point following dextrose gavage by 2-way ANOVA with Bonferroni *post hoc* in H-VSG vs. H-Sham and C-Sham ($N = 3$-10/group). **(G)** Circulating triglycerides following 4 h fast are increased in female offspring of HFD dams receiving VSG ($P < 0.05$) by 1-way ANOVA, ($N = 3$-6). **(H)** Circulating cholesterol following 4 h fast. No significant differences. **(I)** Body weight of postnatal day 60 female rat offspring after maintenance on chow from weaning to postnatal day 60. Female VSG still weighed less (*$P < 0.05$) by 1-way ANOVA, ($N$=3-10). **(J)** An intraperitoneal glucose tolerance test was performed using 1.25g dextrose/kg body weight following an 8 hour fast. No differences between groups existed. **(K**) Body weights for male rat offspring after maintenance on a HFD from postnatal days 60 to 110. No differences between groups existed. Data are presented as mean  SEM, 1 male/litter. **(L)** Body fat composition in female offspring following 4 weeks on HFD. Offspring of VSG-operated rats had greater levels of adiposity (*$P < 0.05$, H-VSG vs. C-Sham), ($N$=3-10). **(M)** Lean body composition percentage. Female offspring of H-VSG dams had less lean body mass composition in comparison to offspring of control dams. (*$P < 0.05$) by 1-way ANOVA, ($N$=3-10). **(N)** Oral glucose tolerance test after 5 weeks on HFD, dosed at 1.25g dextrose/kg average body weight after an 8 hour fast. (*$P < 0.05$) at 15 minute time points following dextrose gavage by 2-way ANOVA with Bonferroni *post hoc*, H-VSG vs. C-Sham. Unique symbol notations (e.g. # vs. †) denotes statistical differences by that variable (diet or surgery). Same symbol notations denote no statistical significance by that variable.