Role of butyrate in restoring satiety signaling in rodent model of high-fat (HF) diet-induced obesity

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Background

- The vagal afferent neural pathway communicates the presence of nutrients in the gut to the brain to induce satiety[1].
- However, chronic ingestion of a high-fat diet (HFD) blunts vagal afferent neuron (VAN) sensitivity to anorexigenic gut hormones, such as cholecystokinin (CCK), resulting in hyperphagia and obesity[1,2].
- In a previous investigation[3], pre-biotics attenuated the deleterious effects of a HFD and increased butyrate concentration in the gut lumen.
- Butyrate, a short-chain fatty acid produced in the gastrointestinal (GI) tract by the fermentation of dietary fiber, may represent a mechanism through which pre-biotics restore gut barrier function and attenuate the obesogenic effects of a HF diet.
- We sought to determine whether butyrate influences food intake, body weight, adiposity, and sensitivity to CCK in the face of a HFD.

The vagal afferent neural pathway

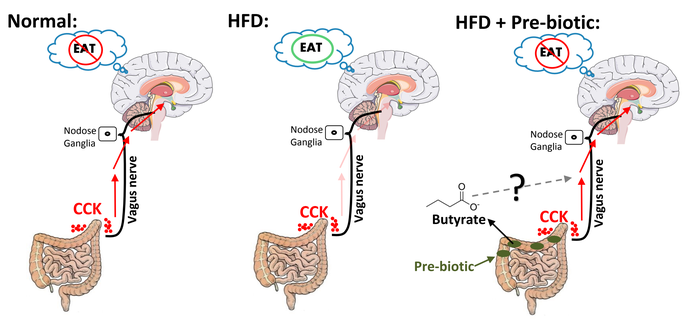
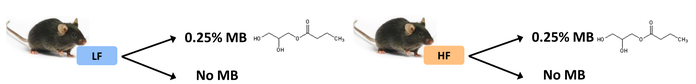


Fig. 1. The vagal afferent pathway, via anorexigenic gut hormones such as cholecystokinin (CCK), communicates the presence of nutrients to the brain to induce satiety. Chronic HFD ingestion causes insensitivity of the vagal afferent neurons (VAN) to CCK, and hyperphagia ensues. Pre-biotic treatment preserves CCK sensitivity in the face of a HFD, and butyrate may represent a mechanism through which pre-biotics influence VAN.

Hypothesis & Methods

Hypothesis: Butyrate preserves CCK sensitivity in the vagal afferent pathway, reduces food intake, and attenuates increases in body weight and adiposity in rodent HFD-induced obesity.

Methods:



- Male C57BL/6J mice (5-6 wk old), in 2 cohorts of 24 mice each, received low-fat (LF) or high-fat (HF) diets for 6 weeks.
- Butyrate was provided as 0.25% monobutyrin (a stable analogue of butyrate) in drinking water to half of each diet group for 6 weeks.
- Food intake (per cage) and body weight were recorded weekly.
- Feeding behavior experiments were conducted in weeks 4 & 6 (Fig. 2).
- Mice were sacrificed for tissue collection at the end of the 6th week.

Feeding behavior experiment timeline

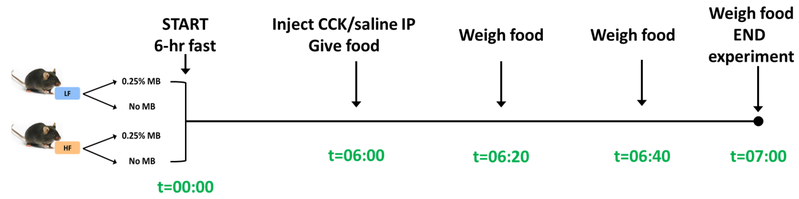


Fig. 2. Outline of feeding behavior experiments conducted in weeks 4 & 6. Mice were fasted in individual housing for 6 hours during the light phase before receiving CCK (3 µg/kg) or saline via intraperitoneal (IP) injection. Immediately post-injection, mice were allowed access to a pre-weighed amount of their respective diets. Remaining food weight was recorded at 20, 40, and 60 minutes post-injection.
Experiments were performed twice during weeks 4 & 6 (each mouse receiving each CCK and saline once) to enable within-mouse comparisons.

Chronic hyperphagia in response to a HFD is unaffected by monobutyrin

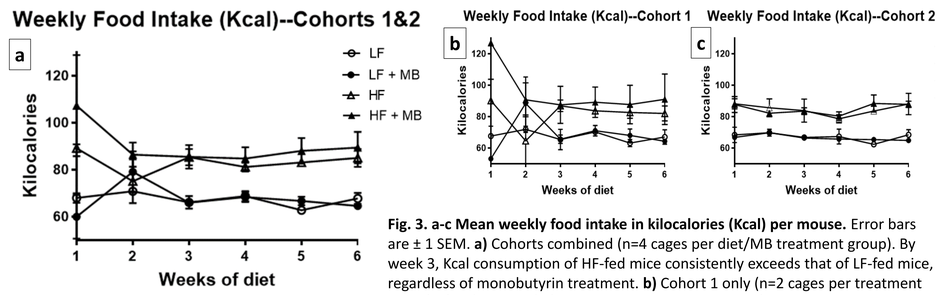


Fig. 3. a-c Mean weekly food intake in kilocalories (Kcal) per mouse. Error bars are ± 1 SEM. **a)** Cohorts combined (n=4 cages per diet/MB treatment group). By week 3, Kcal consumption of HF-fed mice consistently exceeds that of LF-fed mice, regardless of monobutyrin treatment. **b)** Cohort 1 only (n=2 cages per treatment group). **c)** Cohort 2 only (n=2 cages per treatment group).

HFD-induced body weight gain and adiposity are unaffected by monobutyrin

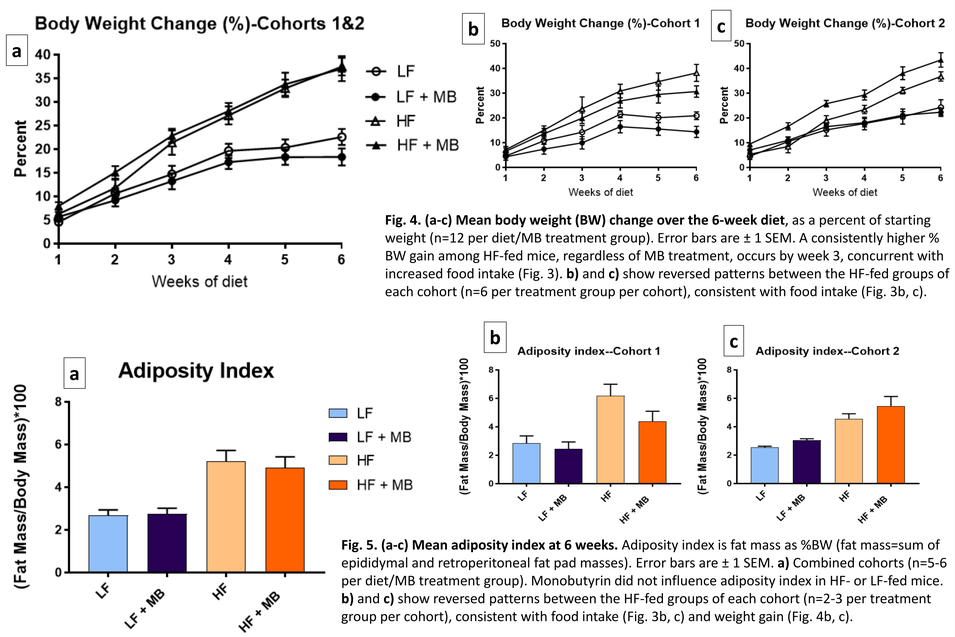


Fig. 4. (a-c) Mean body weight (BW) change over the 6-week diet, as a percent of starting weight (n=12 per diet/MB treatment group). Error bars are ± 1 SEM. A consistently higher % BW gain among HF-fed mice, regardless of MB treatment, occurs by week 3, concurrent with increased food intake (Fig. 3). **b)** and **c)** show reversed patterns between the HF-fed groups of each cohort (n=6 per treatment group per cohort), consistent with food intake (Fig. 3b, c).

Fig. 5. (a-c) Mean adiposity index at 6 weeks. Adiposity index is fat mass as %BW (fat mass=sum of epididymal and retroperitoneal fat pad masses). Error bars are ± 1 SEM. **a)** Combined cohorts (n=5-6 per diet/MB treatment group). Monobutyrin did not influence adiposity index in HF- or LF-fed mice. **b)** and **c)** show reversed patterns between the HF-fed groups of each cohort (n=2-3 per treatment group per cohort), consistent with food intake (Fig. 3b, c) and weight gain (Fig. 4b, c).

Monobutyrin preserves CCK sensitivity during HFD

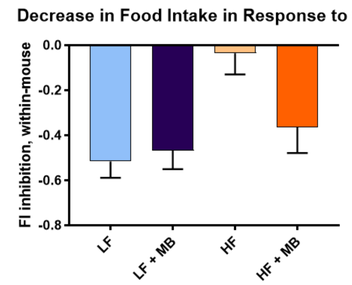


Fig. 6. Inhibition of food intake (FI), within-mouse, in response to CCK injection during week 6 feeding behavior experiments (Fig. 2) (mean ± 1 SEM, n=12 per diet/MB treatment group). FI inhibition is shown as the difference in cumulative Kcal consumption 40 minutes post-injection with saline vs. CCK for a given mouse (plotted on a negative axis). HF-fed, MB-treated mice trend toward greater FI inhibition, thus greater sensitivity to CCK, than HF-fed, non-MB mice (p=0.07, 1-way ANOVA). This effect is consistent across both cohorts.

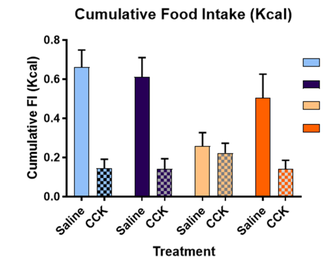


Fig. 7. Cumulative FI in Kcal 40 minutes post-injection with CCK or saline during week 6 feeding behavior experiments (Fig. 2) (mean ± 1 SEM, n=6 per diet/MB/injection treatment group). HF-fed, monobutyrin-treated mice trend toward greater FI inhibition, thus greater sensitivity to CCK, than HF-fed, non-MB-treated mice.

Conclusions

- Ingestion of a HFD increased food intake, body weight, and adiposity as expected, but administration of monobutryin had no effect on these parameters.
- Ingestion of a HFD decreased the ability of CCK to inhibit food intake; however, monobutyrin partially restored CCK-induced FI inhibition.
- Butyrate produces anti-inflammatory effects in the colon via activation of a G-protein coupled receptor, GPR109a[4].
- Vagal afferent neurons express GPR109a, representing a possible direct mechanism of butyrate to influence VAN function.
- Future experiments will seek to determine whether butyrate exhibits protective effects on VAN signaling at the cellular level through CCK-mediated activation of hindbrain neurons.

Acknowledgements & References

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