Chronic High-Fat Diet Impairs Gastric Motor Functions Through Reduced Nitrergic Transmission in Rats

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Background

- Obesity develops as a consequence of insufficient energy expenditure and/or excessive calorie intake.
- Chronically consumed high-fat diet (HFD) has been shown to impair both autonomic vagal and enteric neurocircuitries in rodents.
- Nitric oxide (NO) plays a pivotal role in regulation of gastric accommodative relaxation and sphincter function which is one of the major determinants along with contractility. Recent studies reported the reduced number of nitric oxide synthase (nNOS)-positive neurons in rats fed HFD.
- Furthermore, It was found mice fed with HFD for 12 weeks that gastric emptying (GE) rate was decreased and distension-mediated vagal afferent signaling was disturbed.
- GI motor functions are under influence of dietary nutrient intake and their absorption.
- In fact, the effects of dietary factors, particularly long-term exposure to the certain types of diets, on the enteric nervous system (ENS) phenotype have not yet been adequately documented.

Aim

- The aim of the present study was to investigate the effect of chronic HFD-induced alterations in gastric emptying, gastric smooth muscle contractility and nNOS expression in myenteric neurons using in-vivo and in-vitro techniques.

Methods

Animals

- Experiments were performed on 4-week-old male Sprague-Dawley rats (100–150 g). The experimental approaches used in the present study were approved by the Animal Ethical Committee of Akdeniz University and performed with standard guidelines for care and use of laboratory animals (B.30.2.AKD.0.05.07.00/99).
- Male Sprague Dawley rats were fed with regular diet or a HFD (60% kcal by fat) from 4- to 16-week-old age. Throughout the diet period, body weight progression was monitored weekly.

Gastric Emptying (GE)

- Rats were given a 1.6 g of preweighed pellet following an overnight fasting and euthanized 90 min after the completion of feeding. The gastric contents were collected and dried at room temperature for 72h. Solid GE was calculated according to the following formula:

$$\%GE = 1 - (\text{weight of the dried content} / \text{weight of the pellet}) \times 100$$

In-Vitro Organ Bath Study

- These experiments are designed to evaluate the effects of HFD on smooth muscle contraction. The rats were fasted overnight and anesthetized by urethane (1.25 g·kg^{-1}, i.p). The longitudinal muscle strips were mounted in 20 ml double-jacketed organ baths filled with Krebs buffer solution, the temperature was maintained at 37°C and gassed with 95%O –5% CO mixture. Mechanical activity was monitored through isometrically via force displacement transducers connected to a digital data acquisition system (PowerLab 8SP, AD Instruments, Castle Hill, Australia).
- The gastric tissues (antrum and fundus) were induced with bethanechol to evaluate smooth muscle contraction responses. Dose-response curve for bethanechol (1x10^{-7}-1x10^{-4} mol/L) in the HFD or control group, a gradual increase in concentration was obtained cumulatively when a stable plateau was reached with the previously added concentration.
- Dose-response curves for sodium nitroprusside (1x10^{-7} to 1x10^{-4} mol/L), a soluble guanylate cyclase activator, were generated to determine whether HFD altered the intrinsic muscle properties of the gastric fundus and antrum. Increasing doses of sodium nitroprusside were applied cumulatively on the smooth muscle strips obtained from rats fed regular diet or a HFD.

Gastric Whole-Mount Longitudinal Muscle –Myenteric Plexus(LMMP) Preparation

- In a separate group, rats were sacrificed by cervical dislocation under isofluorane anesthesia. Gastric tissues were removed and placed in a dissecting dish containing phosphate buffered saline (PBS, pH 7.4), opened along the small curvature and greater curvature. After the tissues were then pinned and stretched on a petri dish containing Sylgard. They were incubated and fixed in 4% paraformaldehyde (PFA) for 18-24 h at 4ºC. Following the incubation tissues were rinsed with PBS, then circular muscle layers were removed from the gastric antrum and fundus under a dissecting stereo microscope.

Immunohistochemistry

- The expression of nNOS was quantified in longitudinal muscle-myenteric plexus (LMMP) whole mount preparations in gastric antrum and fundus by immunohistochemistry. The positive cells were counted in 5 ganglia from each preparation and were expressed as number of neurons per ganglia.
- Cuprolinic blue staining was performed to quantify number of the fundic and antral myenteric neurons. The total number of the neuronal cells (Cuprolinic blue-positive cells) in ganglia was also counted.

Results

- Four week-old rats were fed a regular diet or HFD for 8 weeks. The body weight of rats fed with HFD was significantly increased ($p<0.05$) compared with the control group (Fig.1).

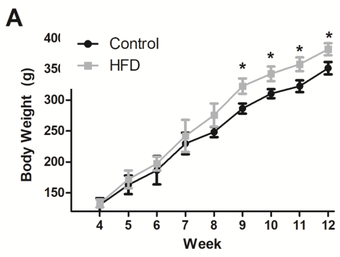

Figure 1. By 12 weeks of age, ingestion of HFD diet rats have higher body weight than ingestion of regular diet rats .n=9 in each group, *p<0.05, Student's t-test.

- Compared with control rats, HFD significantly ($p<0.05$) delayed GE (Fig.2).

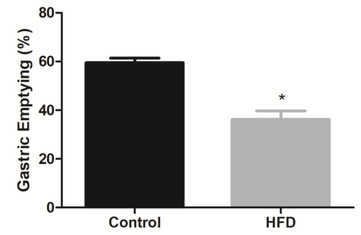

Figure 2. Effect of HFD on solid GE in rats. There was a 23% of delay in GE induced by 8-weeks of HFD compared with control rats fed with regular chow. n=9 in each group, *p<0.05, Student's t test.

- The contraction-responses were evaluated by application of muscarinic agonist bethanechol in HFD (n=7) and control rats (n=5), (Fig.3). Antral (Fig.3A) and fundic (Fig.3B) smooth muscle strips were exposed to the increasing doses (10^{-7} to 10^{-4} mol/L) of bethanechol. As shown in Fig.3C, bethanechol caused a dyscontractility in antral muscle strips obtained from rats fed with HFD compared with the control rats. In fundic muscle strips, contractile responses (Fig.3B) were significantly ($p<0.05$) attenuated in HFD samples with respect to the controls (Fig.3D).

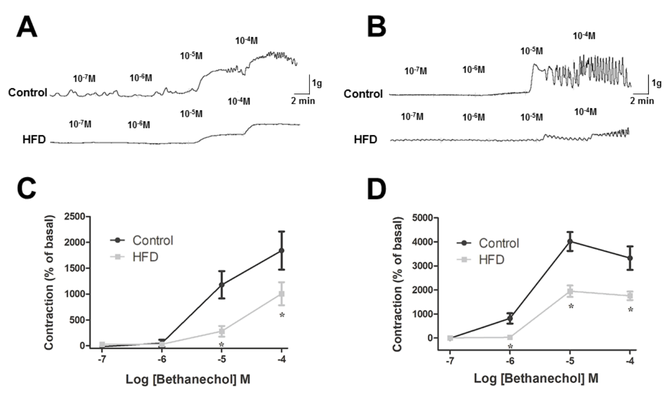

Figure 3. The effect of HFD on gastric contractility in antrum **(A)** and fundus **(B)** preparations treated with muscarinic agonist betanecol. The representative traces demonstrate the contractile response after the application of bethanecol. HFD; n=7, Control; n=5 *p<0.05, Mann Whitney-U test..

- To investigate the HFD-induced changes in gastric relaxation responses, NO donor SNP was used and applied with increasing doses (10^{-7} to 10^{-4} M) on gastric antral (Fig. 4A) and fundic (Fig.4B) smooth muscle strips . We found that the relaxation responses in the gastric fundus and antrum were significantly ($p<0.05$) decreased in HFD rats (n=7) compared with the control rats (n=5)(Fig.4).

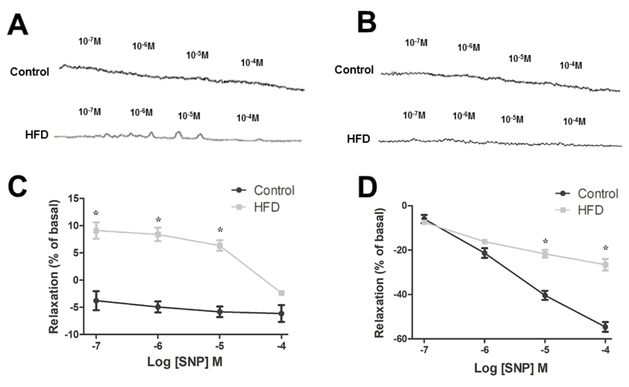

Figure 4. The effect of HFD on gastric relaxation response in antrum**(A)** and fundus**(B)** preparations treated with SNP. The representative traces demonstrate the contractile response after the application of bethanecol. HFD; n=7, Control; n=5 *p<0.05, Mann Whitney-U test..

- To further demonstrate whether HFD alters the expressions of nNOS, in myenteric ganglia, immunofluorescence labeling of nNOS (Fig.5), and neuronal marker PGP 9.5 was performed in antral and fundic whole-mount LMMP preparations. The number of nNOS-positive neurons in antrum and fundus was declined significantly ($p<0.05$) in following HFD regimen (Fig.5B)

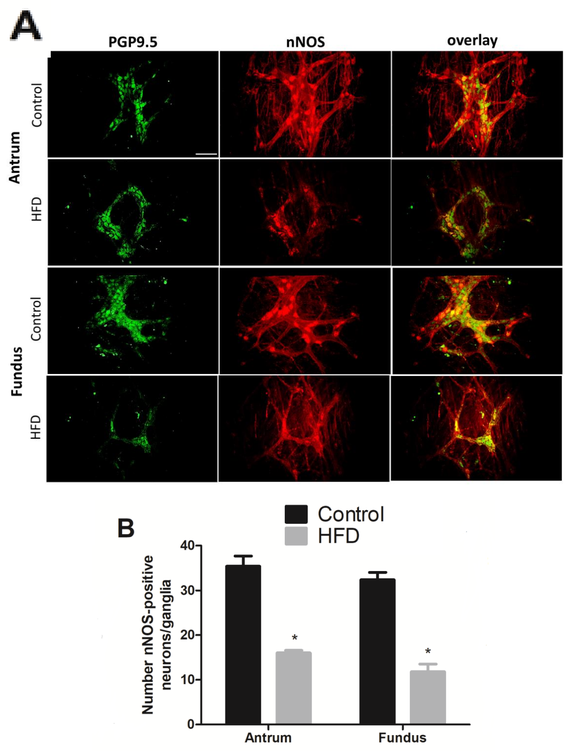

Figure 5. (A) Representative images of PGP9.5 (green) and nNOS-positive (red) in antrum (upper) and fundus (lower) of control and HFD myenteric ganglia. PGP9.5 (green) was used to label the myenteric neurons in the preparations. Scale bar represent 100µm.**(B)** Number of nitrergic (nNOS, B), myenteric neurons in the antrum and fundus of control and HFD rats. n=3, in each group. *p<0.05; non-parametric Mann Whitney-U test.

- We stained neuronal cell bodies to quantify the number of myenteric neurons. To assess the total number of cells in the ganglia, we used immunofluorescence staining protocol of enteric neuronal marker PGP9.5 and histochemical staining of cuprolinic blue(Fig.6). HFD rats showed decreased numbers of myenteric neurons per ganglion in the antrum ($p<0.05$) and fundus compare with control group (Fig.6C).

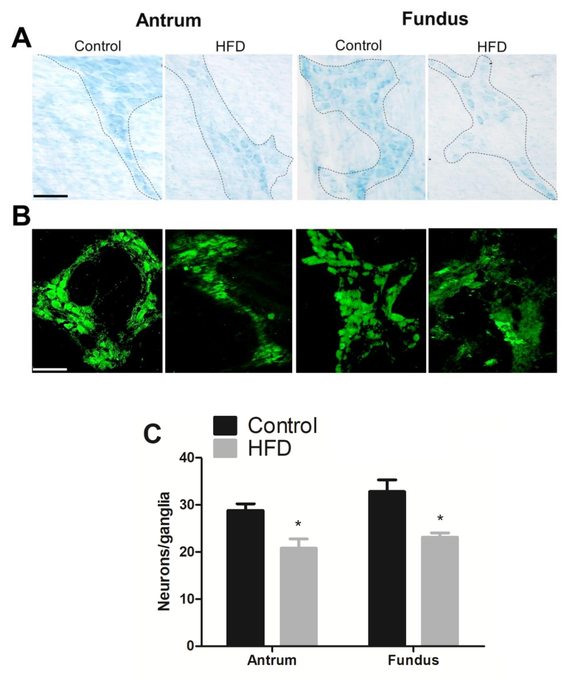

Figure 6. Representative images of neuronal cells in myenteric ganglia stained with cuprolinic blue **(A)**, PGP9.5 **(B)** in the antrum (left) and fundus (right) of control and HFD rats. Cuprolinic blue and PGP9.5 (green) were used to label the myenteric neurons in the preparations. Scale bar represent 50 µm. Number of neurons per ganglia **(C)** myenteric neurons in the antrum and fundus of control and HFD rats. The number of neuronal cells per ganglia was decreased in HFD-fed rats. n=3, in each group. *p<0.05; non-parametric Mann Whitney-U test.

Conclusion

- The present data suggest that chronically exposure to HFD between early adolescence and adulthood results in obesity accompanied by dyspeptic symptoms and impaired gastric motor functions which appear to be mediated by reduced nitrergic neuromuscular transmission.