

Intestinal Electric Stimulation Accelerates Whole Gut Transit and Promotes Fat Excretion in Conscious Rats

Short title: Intestinal electric stimulation and fat excretion

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Grant Support: This work was partially supported by a grant from National Institutes of Health (DK063733)

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Abstract

Introduction: Intestinal electric stimulation (IES) is proposed as a potential tool for the treatment of morbid obesity. Our previous study showed that IES with one pair of electrodes accelerated intestinal transit and decreased fat absorption in a segment of the jejunum in the anesthetized rats. The aims of this study were to assess the effects of IES on the whole gut transit and fat absorption in conscious rats, to examine the effects of multi-channel IES, and to explore the cholinergic mechanism behind the effects of IES.

Methods: Thirty-eight male rats implanted with serosal electrodes were randomized into five groups: control without IES, 2/3 channel IES with short pulses, atropine and atropine plus IES. The whole gut transit and fat remained and emptied from the gut were analyzed after continuous 2-hour IES.

Results: Two and three channel IES significantly accelerated phenol red (marker used for transit) excretion (ANOVA, $P < 0.001$). No significant difference was found between two and three channel IES. Two channel IES significantly increased the excretion of fat ($P < 0.05$). Atropine significantly blocked the accelerated transit induced by IES (ANOVA, $P < 0.001$). Correlation was found between the percentage of phenol red and fat retained in the whole gut ($r = 0.497$, $P < 0.01$).

Conclusions: IES accelerates whole gut transit and promotes fat excrement in conscious rats, and these effects are mediated through the cholinergic nerves. These findings are in support of the concept that IES may be a promising treatment option for obesity.

Key words: intestinal electrical stimulation, electrical pacing, obesity, gastrointestinal motility, nutrition, absorption.

Introduction

Obesity is a major public health problem and is described by the World Health Organization as a “global epidemic”. Extrapolating from the existing data suggests that by the year 2025, up to 50% of the population in the United States will be obese if current trend continues. There is convincing evidence that the intake of high energy dense foods promotes weight gain. One of the most significant promoters of weight gain is fat (1). How to prevent and treat obesity is still a challenge for medical researchers, doctors and the society as a whole.

Gastrointestinal electric stimulation (GES) has been under intensive investigation for its potentials in the treatment of various diseases, such as gastroparesis, nausea and vomiting, short bowel syndrome, and the Roux stasis syndrome (2). Intestinal electric stimulation (IES) has been found to reverse distension-induced intestinal dysrhythmia (3), to accelerate intestinal transit slowed by ileal brake (4). Recent studies have shown that IES activates neuronal activity in the nuclear tractus solitarii (NTS) dependent of stimulation energy (5) and decreases the mean gastric fundus ghrelin level and increases the mean duodenal CCK-8 concentration (6). Reduction in both food intake and body weight with IES is also reported in regular and obese rats (7). These inspiring findings indicate that IES might have a therapeutic potential for obesity. Accordingly, it is of a great interest to investigate if IES exerts any effect on the intestinal absorption.

Our previous study has shown that IES with one pair of electrodes accelerates the intestinal transit measured by the recovery of phenol red, and increases the percentage of triglycerides recovered from the distal cannula in a segmental jejunum of an anesthetized

rat (8). However, it was unknown whether the similar effect could be observed in conscious rats, or the multi-channel IES could be more effective than the single channel IES performed in the previous study. In order to answer these questions, a series of specially designed experiments were carried out in this study. The aims of this study were 1) to assess the effects of IES on the whole gut transit and fat absorption in conscious rats; 2) to examine the effects of the multi-channel IES on the gut transit and fat absorption; 3) to explore the role of cholinergic nerves involved in the effects of IES.

Methods and Materials

Subjects

Thirty-eight male Sprague-Dawley rats (Charles River Lab, MA, U.S.A.) weighing 250-350 g were used in this study. All rats were housed under the temperature and humidity-controlled condition in a 12/12 hours light/dark (06.00 to 18.00 hours) cycle. The surgical and experimental protocols were performed under the approval of the Institutional Animal Care and Use Committee at the Oklahoma City VA Medical Center.

Surgical processes

After intraperitoneal anesthesia with ketamine (60mg/kg) and xylazine (7mg/kg), an abdominal midline incision was made and the antral area of the stomach and small intestine was exposed. One fistula was made at the anterior wall of the stomach, 1 cm from the pylorus. One end of a Silastic tubing (0.012 i.d. x 0.025 o.d., 25 cm) was inserted from the fistula into the duodenum for injecting phenal red (PR) and perfusing

fat solution. Two or three pairs of 28-gauge cardiac pacing wires (A&E Medical, Farmingdale, NJ) were sutured on the serosal surface of the small intestinal segment, 1cm, 21 or 41 cm distal to the pylorus, respectively (Fig 1). The other tips of the Silastic tubing and electrode wires were subcutaneously tunneled through the anterior abdominal wall and threaded subdermally to the neck of the rat. The abdomen was irrigated with 0.9% saline and closed with sutures. Then, the rat was treated with antibiotics (Gentamicin 5 mg, i.m.; Fujisawa USA, Deerfield, IL) for 3 days and housed in a single cage and allowed to recover for 3 to 5 days.

Experimental design

Three to 5 days after surgery, the rats were randomized into five groups (7-8 rats in each): control without IES, two-channel and three-channel IES with trains of short pulses, atropine per se and atropine plus two-channel IES with trains of short pulses. The rats were deprived of food but not water for 18 – 20 hours before the experiment. On the experimental day, the rat was loosely restrained in a Plexiglas tube. Under the conscious condition a bolus injection of 1ml of 5 % phenol red (PR, 0.5 mg/ml) was given as a nonabsorbable marker and then 10 ml 20% Intralipid solution (Sigma-Aldrich Co., St. Louis, MO; purified 20% soybean oil and 1.2% egg phospholipids, 2.2% glycerol anhydrous, pH is approximately 8) was perfused at a rate of 1.0 ml/min for 10 min with a Reglo Digital roller pump (Ismatec SA, Switzerland). In atropine and atropine with IES groups, 0.5 mg/kg of atropine was injected intramuscularly 10 min before the administration of PR. In the IES and atropine plus IES groups electrical stimulation was performed continuously for 2 hours immediately after the ingestion of PR. Two hours

after the ingestion of PR, the rat was sacrificed with overdose of ketamine plus xylazine and cervical dislocation. The stomach, small intestine (divided into equal 6 segments), cecum and colon were removed and their intraluminal contents were flushed with 5 ml 0.15 M saline into separate weighed glass tubes. The content emptied from the anus was also collected. The PR and fat in each of the samples were analyzed and calculated.

Measurement of whole gut transit time

The whole gut transit time was expressed by the percentage of PR retained and output from the gut. PR in each sample (0.3 ml taken from the total amount of each segment content) was measured by adding 1 N NaOH, centrifuging at 1560 *g* for 2 hours in order to separate and remove the fat. A spectrophotometer (Turner[®] Spectrophotometer, SP-830, Apogent-Technologies company, Dubuque, IA, USA) was used to measure absorbance at a wavelength of 560 nm. To calculate the amount of PR in each sample, the concentration determined by the spectrophotometer was multiplied by the weight of each segment. The assessment of phenol red was expressed as the ratio between the collected PR and the total amount of PR injected.

Analysis and calculation of whole gut fat

Fat content in each sample was measured gravimetrically. The sample contents were vortexed with 25 ml of Dole's extraction mixture (9), 15 ml heptane and 10 ml distilled water. Then, 10 ml separated heptane phase was taken and extracted fat (g) was measured in weighed aluminum weigh boats.

The total fat was calculated as the weight (g) of perfused 20% Introlipid multiplied by its fat content (180 mg/g). To calculate the fat retained in the samples, the weight of the extracted fat in the aluminum weigh boats was multiplied by the weight of the each sample content including the 5 ml flushed saline. In addition, the amount of fat in the 0.3 ml content for examining PR was also calculated and added. As there is endogenous fat in the stomach and intestinal tissues, we also performed experiments in 5 rats without Introlipid perfusion to obtain the amount of fat produced endogenously (see Table 1). Consequently, the total collected fat in each rat = extracted fat in the aluminum weigh boats + 0.3 ml content for PR – endogenous fat. The percentage of fat remaining in each segment was defined as the ratio between the total collected fat and the total amount of fat perfused. Fat absorption in the small intestine was assessed as inversely proportional to the amount of remaining fat. In our previous study, fat absorption was assessed as inversely proportional to the recovered triglycerides and fatty acids from the distal cannula (8).

Geometric center

The geometric center (GC) reflects the center of gravity for the distribution of PR and fat within the gut. The GC was calculated based on the amount of PR and fat in each of these 10 segments (stomach as segment 1, small intestine as segments 2-7, cecum as segment 8, colon as segment 9, and output from the anus as 10) as $\sum (\% \text{ PR and fat per segment} \times \text{segment number})/100$ (10).

Intestinal electric stimulation

Two channel or three channel IES was applied simultaneously via the electrodes sutured on the surface of small intestine for two hours. IES with trains of short pulses was chosen in this study because our previous study showed that trains of short pulses were more effective than those of long pulses on the segmental fat absorption in rats (8). The IES with trains of short pulses was conducted using a train of 2s on time, 3s off time, 20 Hz pulse frequency, 2ms pulse width, and 4mA pulse amplitude. The two or three pairs of electrodes received the same electrical stimuli without any time shift.

Statistical analysis

Analysis of variance (ANOVA) was applied to assess the differences in PR and fat remaining in the gut among different groups. Unpaired Student's *t*-test was applied to investigate the difference between two groups. Linear regression was utilized to analyze the relationship between PR and fat from the samples. Statistical significance was assigned for $P < 0.05$. All data are presented as the mean \pm SE.

Results

Effects of IES on gut transit

Comparison between two and three-channel IES

Both two channel IES and three channel IES significantly accelerated PR excretion from the anus, increased the GC and decreased the percentage of PR retained in the whole gut. As shown in Fig. 2, IES with two or three pairs of electrodes significantly accelerated the PR excretion from the anus (ANOVA, $P < 0.001$). The GC of distribution of the PR was

significantly smaller in the control group (GC = 5.01) when compared with IES with two (GC = 6.62) or three pairs of electrodes (GC = 7.45). IES significantly decreased the percentage of PR retained in the whole gut (ANOVA, $P < 0.001$). The percentage of phenol red retained in the whole gut was $97.2 \pm 1.5\%$ in the control group and $70.6 \pm 5.3\%$ in the group of IES with two pairs of electrodes ($P < 0.01$) and $60.3 \pm 7.4\%$ in the group of IES with three pairs of electrodes ($P < 0.01$) (Fig 3). No significant difference was found between two channel IES and three channel IES.

Cholinergic mechanism of IES on transit

The acceleration of gut transit by IES with trains of short pulses was found to be mediated by the cholinergic nerves. Since the effects of IES with two and three pairs of electrodes on the gut transit were similar, we only tested IES with two pairs of electrodes in the group of atropine plus IES. Cholinergic nerve blockage, atropine significantly blocked the accelerated transit induced by IES (ANOVA, $P < 0.001$). The percentage of PR retained in the whole gut was $97.2 \pm 1.1\%$ in the atropine group and was $89.7 \pm 3.3\%$ in the atropine plus IES ($P > 0.05$ vs. atropine alone; $P < 0.05$ vs IES). The percentage of PR retained in the whole gut in the atropine or atropine plus IES groups was comparable to that in the control group ($P > 0.05$, each vs. control) (Fig 3).

Effects of IES on intestinal fat absorption

Endogenous fat in the stomach and intestine

We examined endogenous fat in the stomach, small intestinal segments 1 to 6, cecum, and colon in 5 normal rats without perfusion of the lipid solution and the results are presented in Table 1. The amount of fat in the stomach was 9.1 ± 0.05 mg that was similar to what was reported in the literature (11).

Effects of IES on fat absorption

IES with trains of short pulses significantly increased the excretion of fat from the anus. The amount of fat output from the gut was 0.0 mg in the control group and 16.6 ± 5.1 mg in the IES with 2 pairs of electrodes ($P < 0.05$) (Fig 4). The amount of fat retained in the whole gut was 808.6 ± 73 mg, 581.7 ± 57.2 mg, and 511.6 ± 70.3 mg in the control, IES with 2 pairs and IES with 3 pairs of electrodes (ANOVA, $P=0.01$, Fig 5).

Cholinergic mechanism of IES on fat excretion

Cholinergic nerves were also found to be involved in the fat absorption. Atropine significantly increased the amount of fat retained in the whole gut. The amount of fat retained in the whole gut was 948.4 ± 86.2 mg in the atropine alone group and 903.6 ± 83.1 mg in the atropine plus IES with 2 pairs of electrodes (Fig 5). No significant difference was noted when IES was applied at the presence of atropine. This suggested that the effect of IES on fat remaining in the whole gut was mediated by the cholinergic nerves.

Relationship between intestinal transit and fat absorption

Correlation was found between the percentage of PR and fat retained in the whole gut ($r = 0.497$, $P < 0.01$) (Fig 6). This result suggested that fat absorption induced by IES, at least partially, related to intestinal transit.

Discussion

The present study showed for the first time that IES with short pulse trains significantly accelerated the whole gut transit and fat excrement in conscious rats, and there was no significant difference between 2-channel IES and 3-channel IES. In addition, the cholinergic nerves played an important role in the effects of IES on the whole gut transit and fat excrement.

IES can be divided into forward and backward stimulations depending on the location of the electrodes. The backward stimulation has been reported to delay intestinal transit in canines (12) and in rats (13). Whereas, forward IES reduced the mean transit time of liquids (14), accelerated intestinal transit slowed by fat-induced ileal brake (4) and accelerated the transit and fat absorption in a segment of the jejunum in anesthetized rats (8). Accordingly, forward electric stimulation (proximal electrode pairs were used in case of two-channel IES) was used in this study since our aim was to reduce fat absorption by accelerating intestinal transit. Based on the stimuli, electric stimulation can be categorized into long pulses (in the order of > 50 ms) and short pulses (in the order of μ s and up to 5 ms). It was reported that IES with trains of short pulses accelerated the movement of colonic solid contents (15). In the previous

study with segmental intestinal transit, IES with trains of short pulses was found to be more effective than IES with long pulses (8) and therefore IES with short pulse train was applied in this study. The pulse width was reported to play a crucial role in the treatment of obesity using IES or GES. In a study with GES, a pulse width of 2-3 ms was found to be more effective than the pulse width of 0.3ms (16). In our previous IES study, the pulse width of 2ms was found to be effective in reducing intestinal segmental fat absorption. Accordingly, the same pulse width of 2 ms was used in this study.

Our present data showed that IES with two or three pairs of electrodes significantly accelerated PR excretion from the anus, indicating accelerated transit of the whole gut in the conscious rats. Interestingly, the accelerated effect of IES was consistent with the previous studies performed in a segment of the jejunum in anesthetized rats (8) and physiological slowing of intestinal transit by the fat-induced ileal brake in dogs (4). The mechanism of IES on intestinal transit has not been fully understood. The possible explanations include: IES might stimulate the nerves that innervate the intestine because cholinergic nerves blocker atropine and serosal application of lidocaine on the jejunum inhibited the effects of IES (8). IES might also influence the electric rhythmicity in gastrointestinal muscles, which is driven by interstitial cells of Cajal (ICC) which might result in the change of neurotransmission from enteric neurons (17). A significant postprandial increase of insulin and a decrease of glucagon have been reported in dogs with a Thiry-Vella loop following IES (18). In addition, a possible reduction of intestinal wall tension (or intestinal mechanical resistance) due to changes in neurotransmitters caused by IES (18) might also participate in the mechanism.

This study also showed that IES promoted fat excrement from the whole gut. This finding was in a good agreement with the result from our previous study in which IES increased the fat recovery percentage in a segmental jejunum of anesthetized rats. The result obtained from this study that IES decreased fat absorption might well suggest that IES could be a useful tool for the treatment of patients with obesity.

To answer whether multiple pairs of electrodes or multi channels were more effective in altering intestinal transit and fat absorption, we examined the effects of IES with two pairs and three pairs of stimulation electrodes in the current study. The results indicated no significant difference between two channel IES and three channel IES. A previous study with monometry performed in our lab indicated that one-channel electric stimulation delivered at the proximal intestine altered intestinal motility of the small intestine within a distal distance of 2 meters from the stimulation point (19). Another intestinal motility study using extraluminal strain gauge force transducers showed that pacing a Thiry-Vella loop resulted in a significant reduction of motility of the loop and a similar reduction of motility of the remaining small intestine (18). The similar effects observed in this study between the two and three channel IES also suggested the involvement of the neural pathway.

The mechanistic experiment of this study indicated that the cholinergic nerves played an important role in the accelerated intestinal transit and decreased fat absorption induced by IES. The vagus nerve supplies stretch receptors as well as tension receptors to the wall of the gastrointestinal tract (20). The vagus nerve is also a mediator of the reduction of meal size by

fats (21). Pre-administration of atropine significantly blocked the effect of IES on intestinal transit and fat absorption. In addition, an augmentation of the adrenergic innervation after parasympathetic denervation (22, 23) by atropine might also play a role.

IES promoted fat excrement and this was at least partially attributed to the accelerated transit. A positive correlation was found between the percentage of phenol red and fat retained in the whole gut. Accelerated intestinal transit induced by IES shortened the time of contact between intestinal epithelial cells and the intraluminal fat, which might be partially responsible for the accelerated excrement of the fat solution. Nevertheless, one could not really draw a one-to-one correlation between intestinal transit and fat absorption without further experimental support, since fat absorption following IES might involve complex processes. These processes might include the alteration of the function of the intestinal absorption cells, the change of three distinct proteins related to the intracellular lipid binding protein family which are the liver-type fatty acid binding protein, the intestinal fatty acid binding protein and ileal lipid binding protein, the change of modulation of monoacylglycerol acyltransferase, as well as the change of the local metabolites and neurotransmitters (24, 25).

IES decreased the fat absorption, which is of great clinical importance. This indicated that IES could be used in the treatment of patients with obesity. In previous studies, IES was found to induce gastric distention and the IES-induced gastric distention was one of the main reasons for the reduced food intake in dogs (26), to delay gastric emptying (27) and to reduce food intake and body weight in regular and obese rats (28). The IES-induced reduction in

fat absorption observed in this study added one more rationale for the use of IES to treat obesity.

In conclusion, IES accelerates whole gut transit and promotes fat excrement in conscious rats. The effects of IES are mediated through the cholinergic nerves. The findings of this study are in support of the concept that IES may be a promising treatment option for patients with obesity.

Acknowledgement

We would like to thank Professor Bela Szabo for his advice and technical supports.

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Table 1. Endogenous fat amount in different segments

Stomach (mg)	Small intestine (mg)						Cecum (mg)	Colon (mg)
	Seg 1	2	3	4	5	6		
9.1± 0.05	4.2±0.27	4.0±0.2	4.4±0.08	6.0±0.31	5.8±0.52	5.7±0.44	7.4±0.32	4.5±0.34

Endogenous fat amount in different segments were examined for 5 rats. Values are means ± SE.

Legends to figures

Fig 1. Experimental rat model.

Fig 2. Distribution of PR marker at the end of the experimental period. The percentage of PR for each segment and the mean geometric center for each group are shown as the mean \pm SEM. *: $P < 0.05$; ##: $P < 0.01$ vs. the control group.

Fig 3. Effects of IES and atropine on intestinal transit. Intestinal transit is represented by the percentage of PR retained in the whole gut. Values are the mean \pm SEM. **, $P < 0.01$ vs. the control group ($n = 8$); #, $P < 0.05$ vs. the atropine group ($n = 10$); \$, $P < 0.05$ vs. the atropine plus IES group.

Fig 4. Distribution of fat (mg) at the end of the experimental period. Fat absorption is represented by the weight of the fat retained in and out of the gut. Values are the mean \pm SEM. **, $P < 0.01$ vs. the control group.

Fig 5. Effects of IES and atropine on fat absorption. $P = 0.01$, ANOVA. *: $P < 0.01$ vs. atropine plus IES.

Fig 6. Relationship between gut transit and fat absorption in the groups of control, two channel IES, three channel IES, atropine, and atropine plus IES ($n = 38$). Intestinal transit is represented by the percentage of PR retained in the whole gut. Fat absorption is

represented by the weight of fat retained in the whole gut. Plotted values are mean of the percentage of PR and weight of fat retained in the whole gut. $r = 0.497$, $P < 0.01$.

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Fig 1.

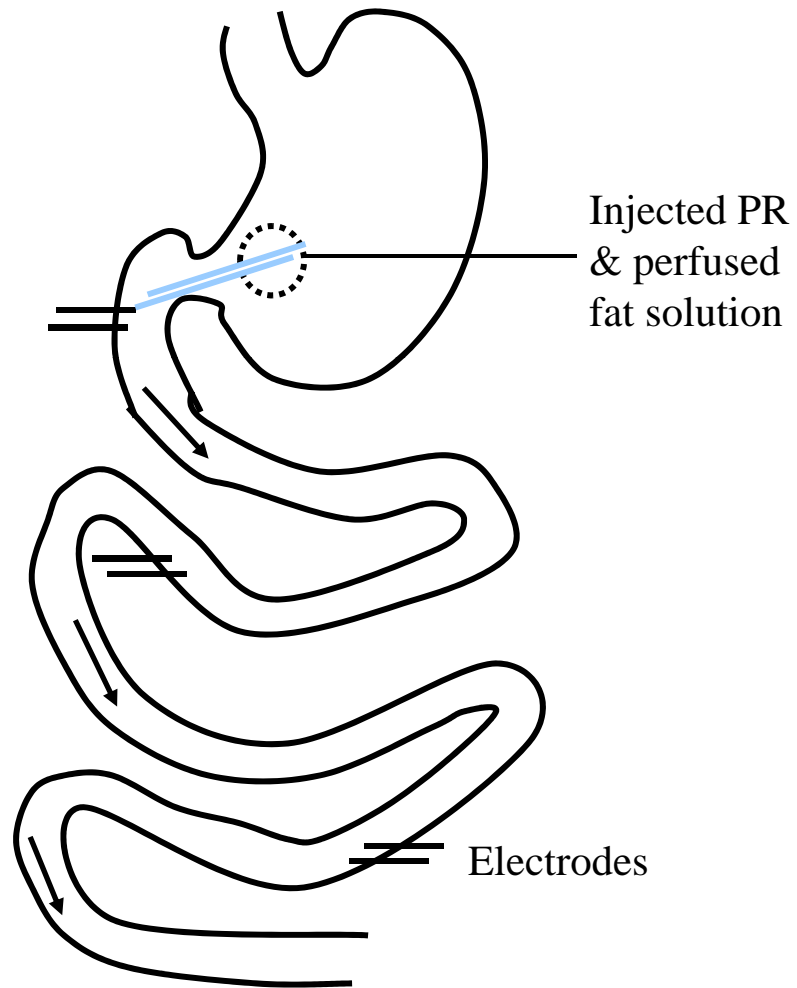


Fig 2.

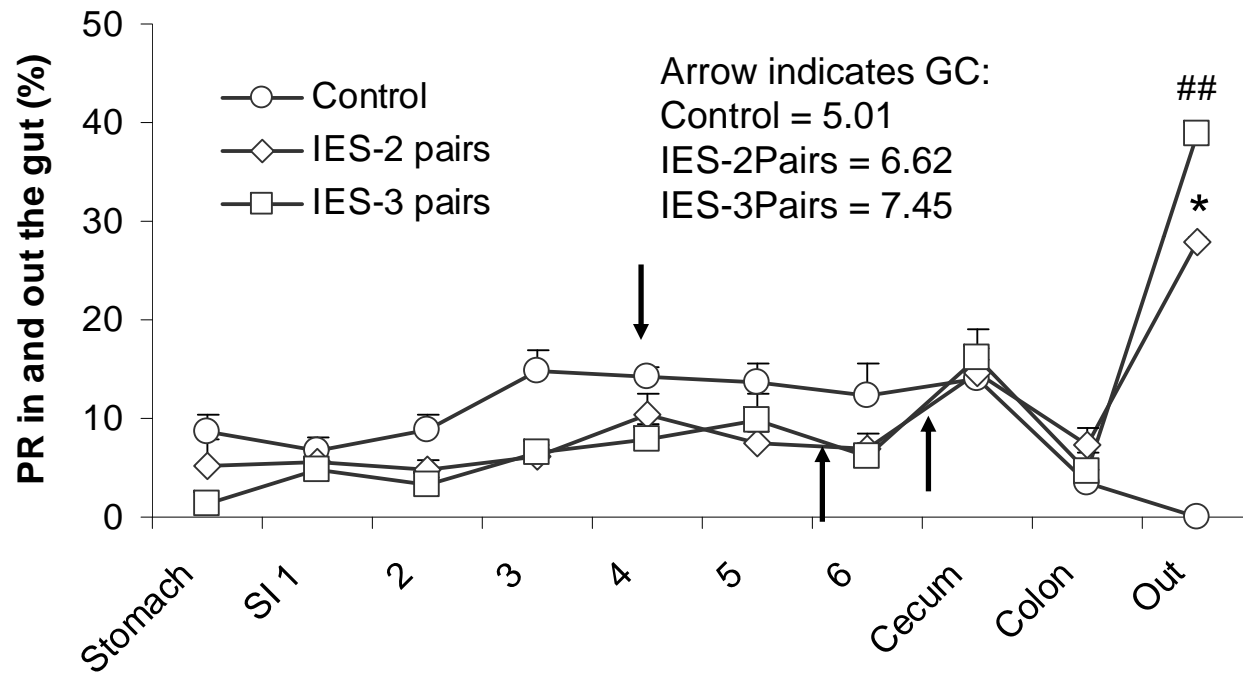


Fig 3.

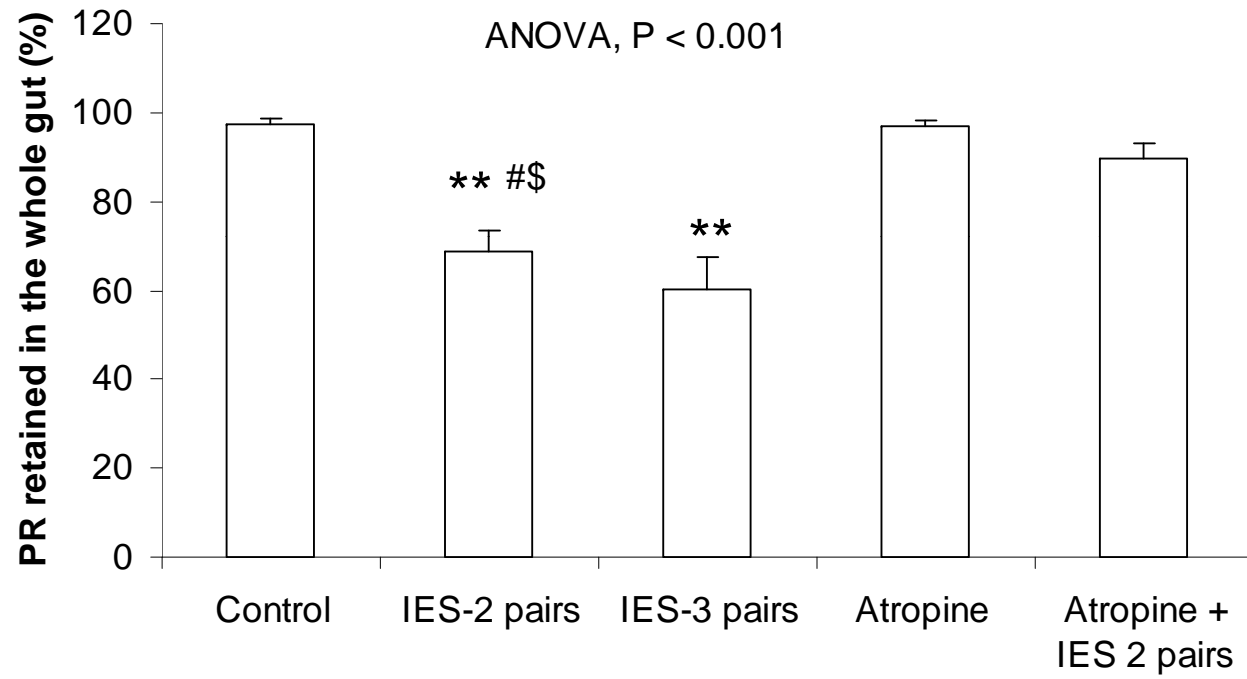


Fig 4.

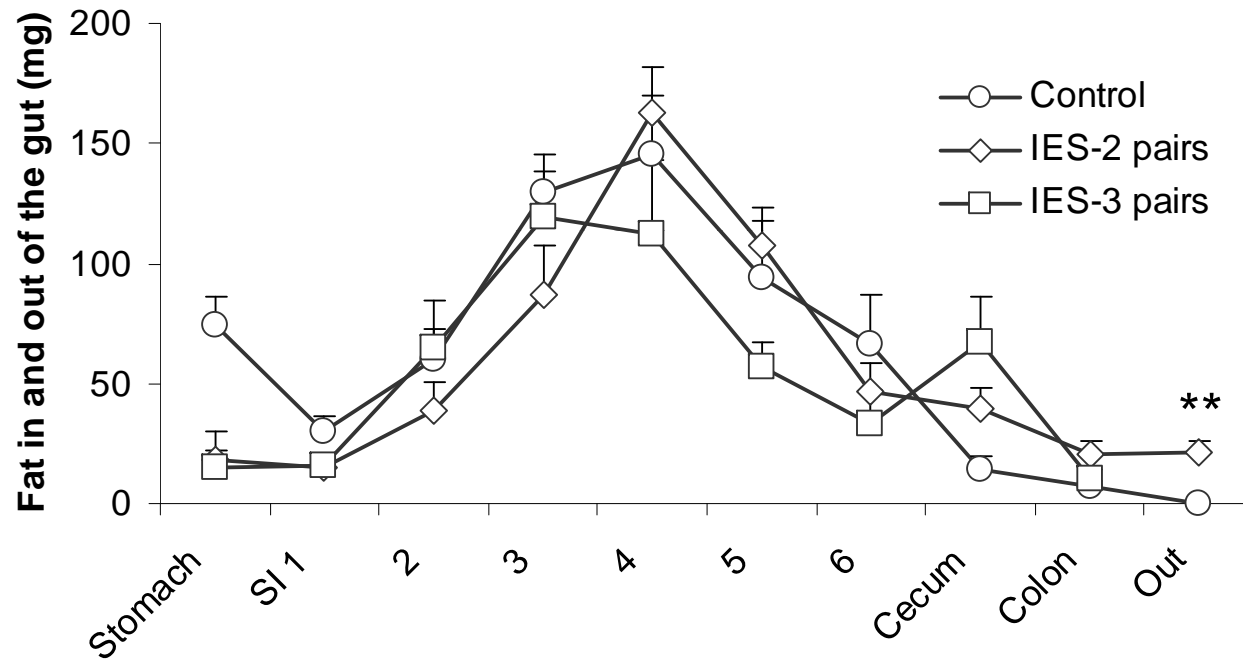


Fig 5.

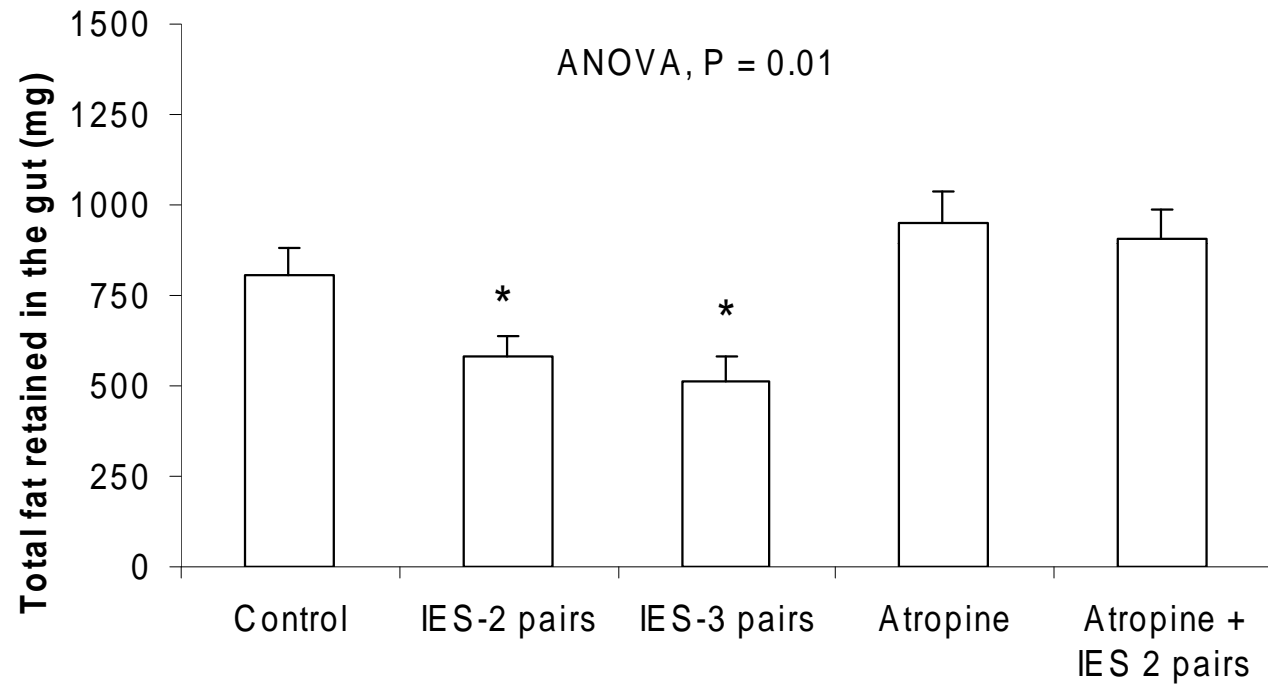


Fig 6.

