Changes in the diet composition of fatty acids and fiber affect the lower gastrointestinal motility but has no impact on cardiovascular parameters: in vivo and in vitro studies.

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Running title:

Effects of FAs and fiber on GI motor and cardiovascular functions

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Abstract

Background: Food and diet are central issues for proper functioning of the cardiovascular (CV) system and gastrointestinal (GI) tract. We hypothesize that different types of dietary FAs affect CV parameters as well as GI motor function and visceral sensitivity.

Methods: Male Wistar rats were fed with control diet (CTRL), diet supplemented with 7% soybean oil (SOY), SOY+3.5% virgin coconut oil (COCO), and SOY+3.5% evening primrose oil (EP) for 4 weeks. The content of insoluble fiber in CTRL was higher than in SOY, COCO or EP. Body weight gain, food/water intake were measured. At day 28 biometric, biochemical, CV parameters, GI motor function (X-ray and colon bead expulsion test) and visceral sensitivity were evaluated. Changes in propulsive colonic activity were determined *in vitro*. The colon and adipose tissue were histologically studied; the number of mast cells (MCs) in the colon were calculated.

SOY, COCO, EP had increased body weight gain but decreased food intake vs. CTRL. Water consumption, biometric, biochemical and CV parameters were comparable between groups. SOY increased the sensitivity to colonic distention. All groups maintained regular propulsive neurogenic contractions; EP delayed colonic motility (p<0.01). SOY, COCO, EP displayed decreased size of the caecum, lower number and size of fecal pellets, and higher infiltration of MCs to the colon (p<0.001).

Conclusions and Inferences: Dietary FAs supplementation and lower intake of insoluble fiber can induce changes in the motility of the lower GI tract, *in vivo* and *in vitro*, but CV function and visceral sensitivity are not generally affected.

Keywords

Key points

1. Laboratory-based studies evaluating the effects of FAs on GI tract motility and pain perception are scarce. The effects may rely on the chain length and type of FAs consumed.

2. Short-term changes in dietary FA and fiber content modified colonic motor function and pain perception, without affecting CV parameters.

3. Type of dietary FA and the amount of fiber impact the function of the GI tract. This should be considered for clinical implications, particularly perioperative, but also in functional GI diseases.

List of abbreviations

ALA – α-linolenic acid

BW – body weight

COCO – SOY diet + coconut oil

CT – total cholesterol

CTRL – control diet

CV – cardiovascular

CVD – cardiovascular disease

DBP – diastolic arterial blood pressure

EP – SOY diet + evening primrose oil

FAs – fatty acids

FI – food intake

GE – gastric emptying

GI – gastrointestinal

HDL – high-density lipoprotein cholesterol

HR – heart rate

IF – insoluble fiber

LCFA – long-chain FAs

MCs – mast cells

PE– phenylephrine

PUFAs – polyunsaturated FAs

SBP – systolic arterial blood pressure

SNP – sodium nitroprusside

TGs – triglycerides

WAT – white adipose tissue

WI – water intake

1. Introduction

The source and composition of fatty acids (FAs) in a diet are important when considering study outcomes and the impact on physiology. Depending on the presence and number of double bonds in the structure, FAs are classified as saturated, monounsaturated or polyunsaturated; within these categories, a particular FA can exert different biological properties. Nutritional guidelines recommend higher intake of polyunsaturated FAs (PUFAs) from plant sources and fish, and lower consumption of trans fat and animal-derived saturated fat [1]. Caution should be taken to the amount and type of FAs consumed since their effect may vary depending on the current physiological state of the body.

As suggested, an adequate supply of long chain FAs (LCFAs) is protective against cardiac mortality and showed an inverse correlation with the risk of coronary events [2–4]. Rangel-Huerta et al. [5] reported that the consumption of n-3 PUFA affects inflammatory biomarkers in cardiovascular (CV) disease (CVD), and acute and chronic conditions. On top of that, the American Heart Association revealed that replacing saturated fat with polyunsaturated vegetable oil can reduce CV events by 30 % [1]. Nevertheless, due to the small number of prospective trials there is an ongoing debate over the practical utility of such dietary regimen in patients with CVD [6–9].

Fiber has long been recommended for the management of various gastrointestinal (GI) ailments, such as constipation or diarrhea; its mechanism of action is well-described elsewhere [10–13]. In contrast, for many years, dietary fat has been studied in non-GI diseases but only recently, the impact of FAs on GI function has become an attractive area of study to combat various GI-related disorders [14–17]. There are some clear signals that dietary FAs are relevant factors in the pathogenesis of GI disorders [16,18–21], but to our knowledge, most results

were based on retrospective or prospective evaluation of dietary questionnaires, which raises the possibility of recall bias. Some studies included biochemical assessment of the level of FAs in patient's serum [22–24]. Once FAs are released in the process of digestion, their presence in the GI tract can be associated with changes in GI function. While evaluating the effects of FAs on GI motility an emphasis should be put on the type and amount of FAs being consumed. FAs undergo different metabolic fates depending on their degree of saturation and the chain length. For example, virgin coconut oil, which recently has gained popularity in the Western diet, is predominantly composed of saturated medium-chain FAs (MCFAs), which are readily absorbed by the enterocytes and transported directly via the portal blood to the liver to produce energy. Unlike MCFAs, long-chain FAs (LCFAs) travel through the lymphatic system via chylomicrons to extra-hepatic tissues where can be stored in the form of triglycerides. Another aspect that should be taken into account is the region and length of intestines exposed to different FAs [25–27]. Few studies evaluated the effects of FAs on the function of the GI tract, i.e. the effect of PUFA on the upper GI motility [28] and visceral pain [29–31], or the effect of saturated FA on the lower GI transit [32].

Since available data cannot unequivocally determine the function of a particular type of FAs in the GI tract, we aimed to evaluate the impact of supplementation with medium- and long-chain FAs on GI motor function, visceral pain and CV parameters with respect to structural changes in selected rat tissues.

2. Materials and Methods

Animals and Diets

A total of 56 male Wistar rats (average initial body weight: 304 ± 4 g for CTRL; 246 ± 4 g for SOY, COCO and EP) were obtained from the Veterinary Unit of Universidad Rey Juan

Carlos (URJC) and housed (4-3/cage) in standard transparent cages (60 x 40 x 20 cm) in a temperature (20ºC) and humidity-controlled room (60%), with a 12 h light/ 12 h dark cycle (lights off between 20:00 and 08.00 hours). The animals had free access to tap water and standard laboratory rat chow (2014 Tekald global 14% protein, Envigo Rms, Barcelona, Spain).

Rats were divided randomly to four dietary treatment groups (14 rats/treatment) for 4 weeks. In the first group, rats were fed with the control diet (CTRL) (2014 Tekald diet); in the second group, animals were fed with AIN93G (SOY) (ZooLab, Poland); in the third group, rats were fed with SOY supplemented with 3.5 % of virgin coconut oil (COCO); in the fourth group, rats were fed with SOY supplemented with 3.5 % of evening primrose oil (EP). In both, third and fourth groups the soybean oil was partially replaced with either virgin coconut oil or evening primrose oil (ZooLab, Poland). SOY, COCO and EP diets contained the same amount of fat but of different origin so that the focus was on the type of FAs rather than on a dosage effect. Soybean oil is rich mainly in PUFAs (approximately 50 % of the FA content) and in monounsaturated FAs (around 35 %); virgin coconut oil contains 70-80 % of MCFAs, in which lauric acid (C12:0) constitutes the majority of the MCFA content. Evening primrose oil in turn contains between 70 and 80 % of linoleic acid belonging to PUFAs and around 10 % of γ-linolenic acid. By choosing natural oils we wanted to reflect the way by which these FAs are supplemented by humans. All diets were formulated to meet the nutritional requirements of growing rats. The ingredients of diets and the content of FAs are listed in Table 1A and 1B, respectively.

After 4 weeks on diet all animals underwent X-ray examination of general GI motor function, and half of each experimental group were used either to *in vivo* colon motor function and

visceral pain evaluations or *in vivo* CV and *in vitro* aorta and colon analyses (Fig. S-1). All animals were kept under their corresponding diet until sacrifice, which occurred within one week after the X-ray study. After visceral sensitivity and CV studies animals were killed by sharp cervical dislocation and exsanguination, and different organs were removed. For the organ bath studies rat aorta and colon were used. In all cases, the investigator was blind to the treatment received by the rat from which the sample under analysis was obtained.

All the experiments were designed and performed in accordance with the European directive and Spanish legislation for the protection of animals used for scientific purpose (EU Directive 2010/63/UE for animal experiments; R.D. 53/2013) and approved by the Ethical Committee at URJC. The animal protocol was designed to minimize pain or discomfort to the animals.

Body weight and food intake

Body weight (BW) was recorded three days a week between 9.00 and 10.00 a.m. during 4-weeks of feeding. Food intake (FI) was measured manually six days a week (except Sundays) between 9.00 and 10.00 am. Remnants of chow were not reused. FI represented the combined data from seven rats separated into two cages belonging to each experimental group and is reported as the average weekly intake normalized to BW. Water intake (WI) was likewise recorded throughout the whole duration of feeding.

2.1 Biometric measurements

The abdominal perimeter and body length (nose-to-anus or nose-anus length) were determined prior to CV studies. The measurements were made in anaesthetized rats (sodium pentobarbital, i.p., 50 mg/kg).

BW and length were subsequently used to calculate the following parameters:

- Body mass index= BW (g)/length^2 (cm^2)

- Lee index= cube root of BW (g) / nose-to-anus length (cm)

2.2 Biochemical determinations and atherogenic coefficient

After four weeks of feeding, and before the CV surgery, rats were deprived of food for 10 hours before the test. Blood samples were collected by tail vein bleeding. The plasma levels of triglycerides (TGs), total cholesterol (CT) and high-density lipoprotein cholesterol (HDL) and glucose were assayed with a portable whole blood analyzer (CardioChek® PA; pts Diagnostics). The collection of blood was performed at 9:00 am in the morning. Additionally, the atherogenic coefficient = (TC – HDL) / HDL was calculated.

2.3 Cardiovascular studies

Blood pressure and heart rate measurements

Blood pressure and heart rate were measured in the anesthetized rats. A catheter coupled to a pressure transducer was inserted into the right carotid artery of the animals for direct measurements of systolic (SBP) and diastolic arterial blood pressure (DBP) and heart rate (HR) using a PowerLab/4e system (PanLab S.L., Barcelona, Spain). Recording of these CV parameters lasted for 10 min [29,30].

Animals were euthanized and used in the following experiments.

Aorta function

Experiment was performed according to the protocol established by Herradón et al. [33]. Briefly, the aorta was excised and placed in ice-cold Krebs-Henseleit (K-H) solution (118

NaCl; 4.75 KCl; 1.2 MgSO$_4$; 1.19 KH$_2$PO$_4$; 2.54 CaCl$_2$; 25 NaHCO$_3$; 11 glucose).

Transverse vascular rings 3–4 mm long were fixed vertically and suspended in a 5-ml

jacketed glass organ bath containing K-H buffer (95% O$_2$, 5% CO$_2$, 37℃). The upper wire

was connected to an isometric force transducer (Grass FT07) and tension measurements were

recorded on a computer (PowerLab/4e program). The rings were mounted with a resting

tension of 2 g and equilibrated for 90 min; the medium was replaced every 15 min. To assess

contractile function, phenylephrine (PE) (10^{-9} M to 10^{-5} M) concentration-response curves

were performed. To evaluate vascular endothelium-dependent- and independent relaxation,

carbachol (10^{-9} M to 10^{-4} M) or sodium nitroprusside (SNP) (10^{-9} M to 10^{-6} M)

concentration-response curves, respectively, were established in PE (1 μM) (submaximal)

precontracted preparations [33]. Only one concentration-response curve was carried out in each

preparation. Contraction responses of the aorta rings are expressed as mean absolute values

and relaxation responses are expressed as the percentage relaxation of the tone induced by

PE.

2.4 Gastrointestinal motor function

Radiographic analysis of the GI tract motor function

GI motor function was studied by plain radiographic methods, as previously described [34,35].

Briefly, X-rays were recorded on Carestream Dental T-MAT G/RA film cassette at different

times (immediately and 1, 2, 4, 6, and 8 h) since contrast administration. The radiographs of

the GI tract were taken using a CS2100 digital X-ray apparatus (Carestream Dental, Madrid,

Spain; 60 kV, 7mA) with a focus distance fixed to 50 ± 1 cm and an exposure time of 0.02 s.

Radiographic images were developed (Kodak X-omat 2000 automatic processor, Kodak AG,

Stuttdart, Germany) and changes in GI motility were semiquantitatively assessed according to

Table S-1. Evaluation was made based on the labelled area of the stomach, small intestine, caecum and the distal colon (fecal pellets) in each X-ray [34–36].

The X-rays were scanned to determine the size and density of stomach, caecum and fecal pellets using an image analysis system (Image J 1.38 for Windows, USA) [37].

Colonic Bead Expulsion Test

Colonic bead expulsion test was performed as described previously [38,39]. Briefly, animals were fasted overnight (for 16 h) with free access to tap water. On the day of the experiment a prewarmed (37°C) glass bead (diameter: 8 mm) with fire polished end was inserted 3 cm into the distal colon using a silicone pusher under light anesthesia [40]. After bead insertion, rats were separated into transparent, individual cages and the time to bead expulsion was measured [41]. Animals were monitored for a maximum of 5 h.

Propulsive motility in isolated rat colon tissue

The entire colon was removed from each experimental group (CTRL, SOY, COCO, EP; n=5-7 rats per group) and set up in a horizontal organ bath (450 mL) to record motor patterns *in vitro*. Before start of the experiment, fecal contents were removed from the preparations by gentle flushing with warmed K-H. The experimental setup was prepared according to previous publications [42,43]. The empty colon was placed into K-H solution (95 % O_2, 5% CO_2, 37°C) and mounted horizontally: the proximal end was connected to a L-shaped plastic connector, using a silk thread, through which warmed K-H solution was infused at 1.5 mL min^{-1} by a peristaltic pump (Masterflex 7815-00, Cole-Parmer, Vernon Hills, IL, USA), whereas the distal end was attached to a T-shaped junction. A pressure transducer (Transpac IV, Hospira, Lake Forest, IL, USA) was connected at the horizontal outlet of the T-shaped

junction to record intraluminal pressure at the anal end; the vertical outlet was attached to an outflow tube through a one-way valve, which prevented the backflow of K-H solution, and simultaneously enabled emptying the colon. The level of the outflow was set to 5 cm above the isolated colon segment to provide a suitable backpressure (corresponding to 5 mmHg). This caused repeated peristaltic contractions in control animals. Outflow was recorded as the weight (1 g=1 mL) of fluid expelled in a beaker located on top of an isometric force transducer (UF1, Force sensor, LCM System Ltd., Newport, UK). Both the anal pressure and outflow were simultaneously recorded under resting conditions and during distension by K-H solution. The signal was acquired using LabChart v6,1.2 software via a PowerLab recording system (ADI Instruments, Oxford, United Kingdom). Activity was recorded by a domestic camera located 40 cm above the organ bath.

Regular peristaltic activity in response to intraluminal fluid infusion was achieved after an equilibration period of 45-90 min. This activity was recorded for 10 min, and then the effects of morphine (10^{-9}–10^{-6} M), and naloxone (10^{-6}–10^{-7} M), were tested on mechanically-induced contractions. Each concentration was allowed to incubate for 10 min. Morphine was used to decrease neuronal contractile activity. Naloxone allowed to revert morphine action. Atropine was added at 10^{-6} M in order to inhibit colonic muscle muscarinic activity. All drugs were added to the organ bath (serosal side).

Different parameters were evaluated during each 10 min-period between doses, as follows: basal pressure (defined as the tonic pressure recorded at the colonic anal end, on top of which phasic changes in intracolonic pressure developed as a result of contractile activity); events per min (frequency of phasic changes in intracolonic pressure exceeding 3 mmHg); peak pressure (defined as the change in pressure associated with the phasic events included in the

analysis of frequency); liquid expelled per event (amount of liquid expelled by each event included in the analysis of frequency); liquid expelled per min (during each 10 min-period).

Each experiment lasted a maximum of 3 h and each preparation was used for a single experiment only.

2.5 Colorectal sensitivity

Four days after the bead expulsion test, we evaluated colorectal sensitivity in the same rats, as previously described [36]. Briefly, each rat was sedated with Sedator® (medetomidine hydrochloride, an alpha$_2$ adrenergic agonist, 1 mL kg^{-1}, 1 mg mL^{-1}, ip) and a 10 cm longitudinal line was drawn over the *linea alba* of the rat abdomen; transverse lines were drawn every 2 cm. Fecal material was removed and a 5 cm long latex balloon lubricated with vaseline was inserted into the colon. The tip of the balloon was 7 cm inside the colorectum. Sedation was reverted with Revertor® (atipamezole hydrochloride, an alpha$_2$ adrenergic antagonist, 0.66 mL kg^{-1}, 5 mg mL^{-1}, ip). The rat behavior was recorded using a video camera (iPad, Apple, Madrid, Spain) located 30 cm below the cage floor. The recording lasted 40 minutes; the first 5 min were discarded; thereafter, the pressure of the intracolonic balloon was increased, using a sphygmomanometer, from 0 to 75 mmHg, in steps of 15 mmHg every 5 minutes, to finally return to 0 mmHg. Pressure was maintained in each 5 min-interval (tonic stimulation).

The videos were exported as series of frames (1 s^{-1}), using the Free Video to JPG Converter program (v.5.0.73). Each frame was analyzed to determine the number and duration of contractions, as well as the percentage of time spent by the rat contracting the abdomen during each 5-min period.

2.6 Histological analysis

Samples from colon and white adipose tissue (WAT) were taken from animals, fixed in buffered 10 % formalin and embedded in paraffin, as previously depicted [36,44]. Briefly, sections of 5 μm were stained with hematoxylin-eosin (HE), toluidine blue or Van Gieson's trichrome stain, and studied under a Zeiss Axioskop 2 microscope (Zeiss International, USA). A qualitative analysis was made in 2 to 4 slices of the colon or WAT per animal. The adipocyte size was measured by counting the number of cells per field under a 20x objective by quintuplicate in 13 to 15 samples per experimental group [44]. The number of mast cells (MCs) was measured after toluidine blue staining under a 40x objective in 10 fields per animal all along the area between epithelium and the external muscular layer [45]; submucosa thickness was measured after staining with Van Gieson's to detect collagen fibers.

2.7 Drugs

Barium sulfate (Barigraf®; Juste SAQF, Madrid, Spain,) was suspended in tap water. Morphine and atropine were obtained from Laboratorios Abelló (Madrid, Spain) and Braun Medical (Rubí, Barcelona, Spain), respectively. All other drugs and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phenylephrine, carbachol, sodium nitroprusside, morphine, atropine and naloxone were dissolved in distilled water.

2.8 Statistical analysis

Statistical analyses and curve–fitting were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). One or two-way ANOVA followed by the Bonferroni's post-hoc test was used for analyses of multiple treatment means. Student's t test was used to compare

single treatment means with control means. The results are expressed as means±SEM. Differences between the means were considered significant when $P < 0.05$.

3. Results

3.1 Effect of dietary intervention on BW, food and water intakes

During the first week of feeding all groups had similar BW gain; throughout the course of the study their BW increased at different rates. A statistically significant change in BW from baseline was observed after 2nd, 3rd and 4th weeks of feeding for SOY, COCO and EP vs. CTRL. Animals were weight-matched at the end of the feeding period and before the planned experiments began (Fig. 1 A) (final body weight: 382 was ± 6 g for CTRL; 382 ± 7 g for SOY, COCO and EP). FI of CTRL group was much higher than the rest of groups; however, the average FI of each experimental group was similar all along the 4 weeks of the study (Fig. 1B). There was a non-significant trend of higher WI for EP-treated animals, particularly at the beginning of the study (Fig. 1C); CTRL, SOY and COCO groups did not show any differences.

3.2. Effects of dietary modifications on biometric and biochemical parameters

The body mass index, Lee index, body length and abdominal perimeter were comparable in all groups (Table S-2). Similar situation refers to plasma glucose, TC, plasma HDL and atherogenic coefficients- all groups produced similar results. The only difference was noted in plasma TG, whose level was significantly increased in EP-treated animals, compared to CTRL-fed rats. SOY and COCO groups had comparable results to CTRL (Table S-2).

3.3. Effect of dietary intervention on CV function

We did not observe any significant differences between CTRL, SOY, COCO and EP groups for SBP, DBP and HR (Table S-2).

Rats fed EP diet exhibited significantly decreased aortic vasoconstrictor response to PE at the concentration of 10^{-6} M, when compared to CTRL-fed rats (Fig. 1D). Neither SOY, nor COCO diets significantly affected this parameter. SOY, COCO and EP diets had no influence on the endothelial-dependent and endothelial-independent responses to carbachol and SNP, respectively (Fig. 1E and 1F).

3.4 Effect of dietary supplementation on GI motility

Radiographic study

Stomach motility curves were similar in all groups, with progressive emptying of contrast throughout time (Fig. 2A). Gastric emptying (GE) was slightly accelerated in EP group, when compared to either CTRL or SOY group, and this effect was statistically significant at time-point 4. In all groups, very little barium remained in the organ after 8 hours.

The maximum contrast capacity in the small intestine was observed 1 hour after contrast administration in all groups (Fig. 2B). All experimental groups showed a typical filling phase from the start point of the experiment to 1 hour after barium administration, and an emptying phase from 1 to 8 hours. The curves obtained from SOY, COCO and EP practically overlapped with those obtained from CTRL at all time points.

No significant differences were noted in the caecum filling phase regardless of the group (Fig. 2C). Eight hours after barium administration the content of the caecum was practically identical in SOY, COCO and EP groups.

In CTRL, SOY and COCO-fed animals, contrast started to be observed in the colorectal fecal pellets 4 hours after its administration and reached the maximum after 6 hours. In contrast, in the EP group, barium was visible in the colorectum from 2 hours after its administration with statistically significant difference compared to CTRL and SOY groups at this time point. After 4 hours, the filling of colorectum with barium in SOY, COCO and EP groups was slower than in CTRL, with significant difference for COCO group (Fig. 2D).

The number of fecal pellets seen in the colon was comparable between groups from the start point to 6 hours after barium administration. After 8 hours, a significant decrease in the number of fecal pellets was reported in COCO and EP groups vs. CTRL (p<0.001 and p<0.05, respectively) (Fig. 2E).

The curves representing stomach area and density (Fig. 3A,B) displayed a similar trend to the semiquantitative analysis performed after X-ray examination. In all experimental groups, the stomach size (i.e. the area of the stomach stained with barium), decreased progressively from the start point of the experiment to 8 hours after barium administration. Only COCO group showed slightly increased contrast density at the start point of the experiment (p<0.05) (Fig. 3B). Two hours after initiation of the experiment, GE was significantly delayed in SOY and COCO groups compared to CTRL (Fig. 4A). Significant difference maintained until 4 hours of the experiment but only for the SOY group; values progressively declined at the following time points. The curve for animals fed with EP overlapped with the curve for CTRL during the whole evaluation period (Fig. 3A,B).

The curves representing changes in the caecum size (the area stained with barium) and density for CTRL-fed animals were similar to the curve from the semiquantitative analysis, but there was a significant decrease in size and increase in density of caecum in animals fed with SOY, COCO and EP diets vs. CTRL (Fig. 3C,D). Eight hours after contrast administration, the areas of caecum for SOY, COCO and EP groups were almost identical, reaching only half the size of that of CTRL (Fig. 4C).

A statistically significant decrease in the size ($p<0.001$) and diameter ($p<0.001$) of fecal pellets and increase in their density ($p<0.01$ for SOY and EP, and $p<0.001$ for COCO) were found in SOY, COCO and EP groups vs. CTRL (Fig. 3E,F); a significant decrease in the diameter of pellets was found for EP vs. SOY group ($p<0.01$).

Colonic motility

Animals fed with SOY, COCO and EP diets exhibited decreased colonic motility *in vivo*, when compared to CTRL. However, a significantly prolonged time to bead expulsion was observed in the EP group, which suggests the strongest inhibitory effect of this diet on propulsive colonic motility (Fig. 4A).

Isolated colons were studied in organ bath at the last day of feeding (Fig. 4B-F). Representative recordings are shown in Fig. S-2. Distention with K-H solution generated anally propagating peristaltic contractions, whose frequencies were similar and changed in a similar manner with morphine, naloxone and atropine in all groups (Fig. 4B). Morphine dose-dependently reduced frequency of events from 0.7-0.9/min to 0.07-0.2/min, naloxone dose-dependently recovered these values and atropine ablated peristaltic activity again.

The values of basal pressure, measured at the distal end of the colon, were significantly higher for animals fed with SOY, COCO and EP diets (around 12 for SOY and 14 mmHg for COCO and EP) vs. CTRL (5 mmHg). The difference was significant for all diets compared with CTRL ($p<0.01$, unpaired t-test before morphine; $p<0.05$ or $p<0.01$, two-way ANOVA, Fig. 4B). Regardless of the diet, increasing concentrations of morphine caused a slight increase in the colonic pressure in all groups; naloxone reduced, and atropine increased again basal pressure. These drug effects were somewhat more remarkable for CTRL and SOY groups (Fig. 4B).

The peak pressure (which corresponds to the intensity of contractions) exhibited a similar trend in all groups. Morphine dose-dependently decreased contractile activity of colonic tissues in all groups, whereas naloxone reversed this effect, with no significant differences between groups (Fig. 4D).

Irrespective of the presence of peaks or the drugs added, liquid expelled per minute was slightly higher in COCO and EP groups vs. CTRL and SOY groups. Morphine did not modify these values, but naloxone slightly increased them in CTRL and SOY (but not COCO and EP) groups. Atropine produced the opposite effect than naloxone in CTRL and SOY groups (Fig. 4E).

The amount of liquid expelled per event was quite similar in all groups before drugs and after morphine at 10^{-9}-10^{-7} M. However, naloxone at 10^{-6} M slightly increased this value in CTRL group, the difference with COCO and EP reaching statistical significance (Fig. 4F).

3.5. Effect of dietary intervention on colonic sensitivity

In CTRL-fed animals there was a progressive increase in the number of contractions and % of time contracting the abdomen in response to increasing mechanical stimulation, i.e. the higher the pressure used, the more contractions were counted (Fig. 5A,C). The duration of the contractions was similar in the course of the experiment (Fig. 5B).

SOY-fed animals also exhibited a progressive increase in the number of contractions, which positively corresponded to increasing intracolonic pressure (Fig. 5A). The area under the curve was higher than in CTRL-fed rats, and showed significantly higher number of events at 15 and 30 mmHg pressures. Although the duration of contractions did not significantly differ from that of CTRL between 0 and 75 mmHg pressure, it significantly increased when the pressure was brought back to 0 at the end of the experiment (Fig. 5B). The % of time with abdominal contractions was higher at 15, 30 and 45 mmHg pressure when compared to CTRL, but only the initial pressure (15 mmHg) induced a significant difference (Fig. 5C).

A graded response in the number of contractions was seen in both COCO and EP groups with a stepwise increase in intracolonic pressure (Fig. 5A). The areas under the curves for both groups were slightly smaller than that of the CTRL group at 60 and 75 mmHg, with no significant differences. At 15 mmHg pressure, both groups had significantly lower number of contractions when compared to SOY group, but only in the COCO group this effect was also observed at 30 mmHg pressure. Significant decrease in the duration of contractions was noted at 60 and 75 mmHg pressures in EP group, when compared to CTRL. Both diets significantly increased the duration of contraction when the pressure returned to 0 mmHg (Fig. 5B). Similar values for the the percentage of time spent by the rat contracting the abdomen were noted for COCO and EP groups (Fig. 5C). A moderate decrease at 60 and 75 mmHg pressures was noted in COCO and EP vs. CTRL; however, when the values were compared to SOY group, both COCO and EP groups exhibited significantly lower values at 15 mmHg pressure. The values for COCO group remained low until 30 mmHg pressure.

3.6. Histopathological analysis of rat tissues after dietary intervention

As shown in Fig. 6A-D, the histological pattern in HE-stained samples of WAT was similar in SOY, COCO and EP groups, and comparable to CTRL group. The quantitative analysis showed a similar density of adipocytes in CTRL- and COCO-fed animals. SOY- and EP-treated rats demonstrated lower number of adipocytes, but the difference was statistically significant only for EP vs. CTRL and COCO (Fig. 6E).

No significant structural modifications were found between groups in sections of the distal colon (Fig. S-4). The average number of mast cells/mm^2 between the epithelium and external muscular layer of the colon was significantly higher in SOY-, COCO- or EP-fed animals vs. CTRL-fed rats. A significant increase in the number of MCs, which infiltrated the colon, was found for EP vs. SOY diet, as well as COCO diet (Fig. 7E).

4. Discussion

In the present study, we verified the effects of diets, which varied in the content of FAs and fiber, on the risk of CV events, GI motility and visceral pain *in vivo*.

Our study shows that SOY, COCO and EP groups had significantly increased BW gain and decreased FI vs. CTRL along 4-weeks of feeding. However, it needs to be emphasized that differences in BW and FI observed between CTRL and the rest of groups could be related to higher initial BW of CTRL animals and lower energy density of their diet. The final BW of animals in all groups were similar. Therefore, the results are consistent with previously reported outcomes, where the growth characteristics and FI remained unchanged during the 4-week period of feeding with either soybean oil [46,47], coconut oil [48–51] or evening primrose oil [52,53].

The cornerstone of current dietary advice is the recommendation to reduce the intake of saturated FAs and replace it with PUFAs, as a means of decreasing the risk of CVD [54]. However, several meta-analyses of observational studies have consistently indicated that the intake of saturated FAs is independently associated with cardiac function, and that current evidence does not clearly support the CV guidelines [54,55]. Some studies of clinical endpoints suggest that PUFA, and α-linolenic acid (ALA) in particular, can have CV benefits; however, overall evidence remains mixed and inconclusive [7,56,57]. Virgin coconut oil is one of the plant products, which proved to have a beneficial impact on the CV system, by preventing blood pressure from rising and normalizing the lipid profile [58], when compared to other oils such as copra oil [59] or sunflower oil [60]. In our study, except for the EP group, that exhibited a significantly elevated level of TGs, the diets did not influence the biometric, biochemical and CV parameters, which were within the normal range of variation (similar to CTRL). The differences between our outcomes and other studies might be related to a different experimental design, particularly the duration of feeding. Within the above-mentioned studies, the oil type and the content of FAs in the respective control group varied. Studies that compared the effects of oils to a control diet incorporated similar lipid content as our CTRL diet and did not show any significant alterations in the biochemical or CV parameters [47,50,58,61].

The function of WAT and colon can be affected by changes in dietary fat intake. Both SOY and EP diets displayed cellular hypertrophy, compared to the CTRL group, shown as a decrease in the number of adipocytes. It is worth mentioning that in SOY, as well as in EP diets, the predominant type of FA belongs to the long-chain PUFA, which can effectively regulate body fat by increasing the volume of adipocytes [62,63]. As previously mentioned, the

EP group had elevated level of TGs in the serum. Indeed, an increased deposition of LCFAs in WAT, principally in the form of TGs, can result from increased LCFA synthesis or uptake, augmented conversion of LCFA to TGs, reduced LCFA oxidation or changes in the TG lipolysis which in turn decreases TG or LCFAs removal [64,65]. Various studies have previously reported the anti-proliferative effects of LCFAs on pre- and adipocytes [66]. Moreover, increased adipogenesis may depend on the uptake of circulating FAs, which in turn can be associated with the presence of ALA in the EP diet [66]; ALA depresses the proliferation of preadipocytes and promotes *de novo* lipogenesis [67].

Every tested oil reaches the GI tract and can affect its function. The initial stomach size values were normal, and its emptying was progressive in all intervention groups. The variations found between SOY and COCO vs. CTRL diets in GE were negligible and likely not clinically relevant. Similarly, the function of the small intestine and caecum were not affected by SOY or COCO diet. The slightly faster GE in the EP group might imply faster propel of the content in the GI tract. In fact, barium reached the colon faster in the EP group, but it filled the organ at a similar rate to other groups. In all groups in which soybean oil constituted the basal fat content, we observed a remarkable reduction in the size of the caecum and significantly higher content density (barium was more concentrated), when compared to CTRL. Not only the higher amount of lipid content in SOY, COCO or EP diets could account for changes in the size of the caecum, but also the lower amount of insoluble fiber (IF) in each diet vs. CTRL. Since IF should resist fermentation and remains relatively intact while passing through the gut, it is unlikely that the observed changes in size of the caecum were associated with altered intestinal gas production. Higher content of IF in CTRL increased the filling of the colon with soft and wider stools, thus producing higher mechanical stimulation and greater influx of water and mucous [68]. Consequently, the

exposure of the stool to the mucosa, without the presence of IF (or not enough amount of IF in a diet) could result in stool dehydration and increase in stool viscosity, which together evoked changes in the gut. In our study, lower amount of fiber in SOY, COCO and EP diets was not accompanied with higher intake of water that could ease the propulsion of the colonic matter. We observed a significantly lower number of stained fecal pellets in the colon in COCO and EP groups vs. CTRL, and a significant decrease in their size and diameter with concomitant increase in their barium density. The results indicate that the fecal matter in SOY, COCO and EP groups was less hydrated than in CTRL group and delayed the passage of stools through the gut. This is in accordance with the outcomes obtained in the colonic bead expulsion test, where SOY, COCO and EP animals had significantly prolonged colonic transit (with the most noticeable change seen in the EP group). Since the morphology of the colon, including the evaluation of the submucosa thickness (Fig. S-4), did not differ between groups, we presume that delay in the pellet formation in SOY, COCO and EP groups was caused by either the lower content of fiber, increased content of lipids or deregulated influx of water to the colon. Further studies should evaluate changes in colonic permeability and the measurements of urinary electrolytes in order to exclude the impaired ion transport through the intestinal wall, which controls the influx/efflux of water from and to the intestinal lumen.

The underlying mechanism for the effects of dietary fiber on GI transit has already been described in many *in vivo* and clinical studies [68]. Although several studies critically assessed the influence of lipids on GI motility; most of dietary interventions incorporated the intake of a high fat diet, which likely attenuates the motile function of entire GI tract [69]. Undoubtedly, some of the effects reported in our study can be attributed to the content of IF. However, since differences in the motility were seen not only between the oils and the control group, but also between each dietary interventions (especially in the colonic bead expulsion test) we

presume that the type of FA in each diet contributed to these effects. To our knowledge, this study has demonstrated for the first time the effects of MCFA and LCFA on GI transit *in vivo*. Whether both types of FAs can constitute a primary causal factor for abnormal propulsive function of the gut is still unknown. It can be speculated that by prolonging the time of feeding or incorporating higher concentration of FAs, the effect on GI transit would be clearer.

Regardless of the diet, all colonic samples were characterized by regular propulsive neurogenic contractions. The neurogenic mechanism worked properly to sufficiently propel the content in the colon, and drugs used in the experiment exerted expected effects (i.e. morphine caused the relaxation of the colon by incrementing the basal pressure and ablating neurogenic contractions, naloxone reversed this effect and atropine relaxed the colon again). The observed marked increase in the basal pressure at the anal end in SOY, COCO and EP groups, may result from changes in the colonic wall that arose during the feeding period and resulted in an adapted response to lower mechanical stimulation. CTRL-fed animals had to response to a larger volume of the fecal matter and thus the colonic wall was probably more relaxed and could more easily adapt to the amount of fluid infused during the in vitro study (reaching lower basal pressure at the distal end). In fact, the distal colon showed a higher diameter for CTRL than for the rest of groups (Fig. S3). The fecal pellets expelled by SOY, COCO and EP-fed animals were smaller and less hydrated than CTRL-fed animals; required greater pressure to propel the fecal matter through the colon. Consequently, the same amount of liquid infused to the lumen could not be accommodated as well as in case of CTRL animals. This might contribute, at least in part, to the differences seen in basal pressure. In contrast, the lower diameter of proximal colon measured before drugs for CTRL diet vs the other groups (Fig. S3A) might reflect a higher tone derived from the higher need to respond

to a bigger volume of fecal matter delivered by the caecum. Even if no structural alterations of the colonic wall were apparent, a more accurate and thorough analysis is necessary to clarify the mechanisms involved.

Few studies evaluated the effects of intraduodenal lipid infusion to humans, either to control individuals [26,28] or patients with irritable bowel syndrome [25,70,71]; however, most of them focused on the upper, rather than the lower part of the GI tract. Moreover, they incorporated the administration of an emulsion, called Intralipid®, composed of LCFAs, which was delivered in different concentrations. Depending on the dose, lipid modulated conscious sensation in a different manner [25]. Increasing the dose resulted in increased sensitivity of the stomach to gastric distension, whereas the lowest lipid dose caused desensitization [25]. Although gastric distension induces conscious sensation, the nature of these sensations and the threshold level are more likely to be modulated by duodenal lipid [25]. Here, we showed that minor changes in the composition of dietary FAs can induce slight, but relevant changes in visceral perception in rats. We observed that in all groups abdominal contractions increased in a pressure-dependent manner; however, SOY diet induced a significant increase in the abdominal contractions, particularly at lower pressures, suggesting that animals fed with SOY were more sensitive to colorectal distensions than rats from other groups. A fairly homogenous duration of contractions, oscillating between 0,94 and 1,27s, was reported in SOY, COCO and EP groups, which indicates that experimental diets had no influence on abdominal muscle motor function. COCO and EP diets significantly inhibited colonic sensitivity to mechanical stimulation at the lowest pressure values. Generally, rats fed with SOY, COCO and EP diets seemed to be more sensitive than the CTRL group, particularly regarding the duration of contraction at the end of the experiment. It is thus possible that the supplementation with the oil rich in MCFAs or LCFAs (particularly ALA) can attenuate the

visceromotor response to colorectal distention in low values. Simultaneously it can enhance and maintain colonic pain perception longer than a diet without additional FA supplementation.

Of all immune cells, MCs are proposed to play a pivotal role in the development and exacerbation of visceral hypersensitivity [72]. In our study, although SOY, COCO and EP groups exhibited greater mast cell infiltration to the colon, their presence was not associated with marked changes in abdominal contractions in response to colorectal distention. Maybe the method of staining we used was unable to detect and distinguish the non-activated form of MCs from their activated, degranulated form. It is worth mentioning that the reaction from motor neurons secondary to degranulation may provoke hypersecretion and power propulsion, leading to diarrhea and abdominal pain [73]. In our study neither accelerated intestinal transit, nor hypersensitivity were reported. Thus, alterations observed in motility or colonic sensitivity studies were not related to the higher number of MCs in the colon. The number of MCs may not be the key factor affected by exposure to FAs. However, the possible explanation may rely instead on the activation status of MCs or their interaction with neuronal endings. Studies on mucosal innervation (e.g. close apposition of mast cells to colonic nerve fibres), would aid in explaining the mechanism responsible for their infiltration after feeding with MCFAs and LCFAs. The extent of changes in FA intake and their relationship with the infiltration of MCs in the colon and visceral sensitivity has not been investigated in detail so far.

To conclude, *in vivo* short-term dietary supplementation with MCFAs or LCFAs in rats: a) does not affect biochemical and CV parameters; b) elicits changes in the size of caecum; c) decreases the number and size of fecal pellets; d) delays colonic transit; e) has no influence

on abdominal muscle motor function; and f) promotes infiltration of mast cells to the colon. Our findings show that changes in the content of FA and insoluble fiber in a diet, do not affect the CV system but can impact the function of the GI tract. The findings emphasize the necessity to control diet for clinical implications, particularly perioperative, but also in functional GI diseases.

Conflicts of interest

The authors have no competing interests.

Author Contributions

PM, RA, VLM and JF provided the overall concept and designed the research study; PM, AG, MMR, VLM, EH, JAU and GV conducted experiments; PM, RA, VLM, EH, AG, MMR, ASY analyzed the data. PM and RA wrote the manuscript. MIMF contributed financial support and essential intellectual input. All authors regularly discussed the experiments and data, suggested adjustments of the experimental protocols, read and approved the final version of the manuscript.

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Supplementary Material

Figure S-1. A schematic representation of the experiments included in the study.

Figure S-2. Representative recordings of the colonic propulsive activity after dietary feeding with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP). Abbreviations: A, atropine; NX, naloxone. Scale bar: 10 min.

Figure S-3. The effects of dietary interventions on the diameter of the proximal (A), mid (B) and distal (C) colon. (D) Representative frame from a video recorded from a CTRL-fed rat colon during organ bath experiment (see main text for details regarding organ bath experiments). Briefly, movies of colonic motor patterns were recorded with domestic camera, positioned above the preparation. Fluid was infused from the inlet located at the proximal end (top). The contents exit the colon with each peristaltic contraction through the outlet, located at the distal end (bottom). The recordings were resembled down to obtain images (frames) per second. Diameters were measured, using Image J, at three different positions: P-proximal, M-mid and D-distal (1 cm from proximal or distal end, and at the mid-point of the colon). The diameter at each point along the length of the colon was calculated for 5 different frames (at least one second apart) belonging to one animal. Frames in which peristaltic contractions were on-going, have not been taken into account. Results are shown as the mean ± SEM (n = 4-7 animals per group). *p<0.05 vs. CTRL (two-way ANOVA followed by Bonferroni post-hoc test).

Figure S-4. Effects of dietary interventions on the general structure of the colonic wall. Animals fed with CTRL (A), SOY (B), COCO (D) and EP (E). Van Gieson´s trichrome stain. Scale bar: 100 µm.

Table S-1. Semiquantitative analysis of the alterations in GI motility based on X-ray images. Each parameter was scored and summed for further analysis [31].

Table S-2. Effects of dietary interventions on biometric, biochemical and cardiovascular parameters.

Figures

Figure 1. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on body weight (A), food intake (B), water intake (C) and aorta function: (D) concentration-response curve of phenylephrine (10^{-9} – 10^{-5} M), (E) concentration-response curve of carbachol (10^{-9} – 10^{-4} M) and (F) concentration-response curve of sodium nitroprusside (SNP) (10^{-9} – 10^{-6} M). Results are shown as the mean ± SEM, n=6-14 rats per group. *p<0.05, **p<0.01 and ***p<0.001, vs. CTRL (two-way ANOVA followed by *post-hoc* Bonferroni multiple comparison test).

Figure 2. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on gastrointestinal motor function and the number of fecal pellets in rats. Gastrointestinal motility was evaluated by radiological methods in: (A) stomach (gastric emptying), (B) small intestine, (C) caecum and (D) colorectum. (E) represents changes in the number of fecal pellets, whereas (F) shows representative X-rays obtained from rats, 2 and 8 h after intragastric administration of barium contrast (2.5 mL, 2 g/mL). Results are shown as the mean ±SEM (n=10-14 rats per group). *p<0.05, **p<0.01 and ***p<0.001, vs. CTRL; ##p<0.01 and ###p<0.001 vs. SOY (two-way ANOVA followed by Bonferroni post-hoc test). Scale bar: 3 cm.

Figure 3. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on gastrointestinal motor function in rats - morphometric and densitometric studies. Morphometry and densitometry analyses of the stomach (A, B), caecum (C, D) and fecal pellets (E,F). The analysis was performed using X-ray images taken immediately and after 1,

2, 4, 6, and 8 h from time of contrast administration (Image J 1.38 for Windows, National Institute of Health, USA, free software: https://rsb.info.nih.gov/ij/). Results are shown as the mean ± SEM (n=10-14 rats per group). $^*p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$, vs. CTRL; $^{##}p<0.01$ vs. SOY (two-way ANOVA followed by Bonferroni post-hoc test).

Figure 4. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on (A) colonic motility assessed in colonic bead expulsion test (n= 6-7 rats/group) and (B-F) on propulsive activity of the isolated colon (n= 5-7 rats/group) . Results are shown as the mean ± SEM). $^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. CTRL (two-way ANOVA followed by Bonferroni post-hoc test). Abbreviations: A, atropine; NX, naloxone.

Figure 5. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on abdominal contractions in response to intracolonic stimulation in rats. (A) number of abdominal contractions, (B) duration of abdominal contractions and (C) percentage of time with abdominal contractions. An intracolonic balloon was used to mechanically stimulate the colon at increasing pressures, for 5 minutes each pressure (tonic stimulation). Results are shown as the mean ± SEM (n = 6-7 animals per group). $^*p<0.05$ and $^{**}p<0.01$, vs. CTRL; $^#p<0.05$ and $^{##}p<0.01$, vs. SOY (two-way ANOVA followed by Bonferroni post-hoc test).

Figure 6. Representative images of histological samples of adipose tissue (A-D) and (E) the number of white adipocytes in the histological sections of epididymal adipose tissue (per area) of rats fed with experimental diets. Experimental groups: (A) control diet (CTRL), (B) AIN93G (SOY) diet, (C) SOY diet supplemented with coconut oil (COCO) and (D) SOY diet supplemented with evening primrose oil (EP). Results are shown as the mean ± SEM (n =

13-14 animals per group). *p<0.05 vs. CTRL; [&]p<0.05, vs. COCO (Student's t-test). A-D: Scale bar 100 µm.

Figure 7. Effect of dietary supplementation with (A) control diet (CTRL), (B) AIN93G (SOY), (C) SOY diet supplemented with coconut oil (COCO) and (D) SOY diet supplemented with evening primrose oil (EP) on distribution and (E) average number of mast cells between the epithelium and external muscular layer (arrows) of rat colonic samples. Results are shown as the mean ± SEM (n = 4-7 animals per group) ***p<0.001 vs. CTRL; ^{###}p<0.001 vs. SOY; [&]p<0.05 vs. COCO (Student's t-test). Scale bar 100 µm.

Tables

Table 1. Ingredient and composition of experimental diets (A), and the type and content of fatty acids (B).

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Table 1.

A) Ingredient and composition of experimental diets.

Ingredients	Nutritional composition of diets (%)			
	CTRL	SOY	COCO	EP
Carbohydrates w/o IF	53,69	62,95	62,95	62,95
- Corn starch	ns	39,75	39,75	39,75
- Sucrose	ns	10,00	10,00	10,00
- Maltodextrine	ns	13,20	13,20	13,20
Insoluble Fibre (IF) [a]	20,13	5,00	5,00	5,00
Proteins	16,00	20,00	20,00	20,00
Minerals [b]	5,26	3,50	3,50	3,50
Vitamins	<1	1,00	1,00	1,00
L-cystine	0,30	0,30	0,30	0,30
Choline hydrogen tartrate	0,10	0,25	0,25	0,25
Tert-Butylhydroquinone	ns	<0,01	<0,01	<0,01
Lipids	4,47	7,00	7,00	7,00
Energy density (kcal/g)	2,9	4,029	4,029	4,029

B) Fatty acid composition of experimental diets.

Type of FA	Content of FA in a diet (%)			
	CTRL	SOY	COCO	EP
Saturated MCFAs	12,5	9,7	69,4	4,0
Saturated LCFAs	2,5	5,1	21,0	1,0
Monounsaturated LCFAs	17,5	23,0	6,7	5,0
Polyunsaturated LCFAs	52,5	54,4	1,8	80,0
Others	15,0	7,8	1,1	10,0
TOTAL	100	100	100	100

[a] IF corresponds to neutral detergent fibre in CTRL diet (neutral detergent fibre is an estimate of insoluble fiber, including cellulose, hemicellulose, and lignin) and to cellulose in purified diets (SOY, COCO, EP). [b] Ash values for CTRL diet, and mineral mix values (AIN-93g-MX) for purified diets. COCO: SOY supplemented with 3.5 % of virgin coconut oil CTRL: control diet; EP: SOY supplemented with 3.5 % of evening primrose oil; FA: fatty acid; IF: insoluble fibre; LCFAs: long-chain FAs; MCFAs: medium-chain FAs; ns: non specified. SOY: AIN93G diet.
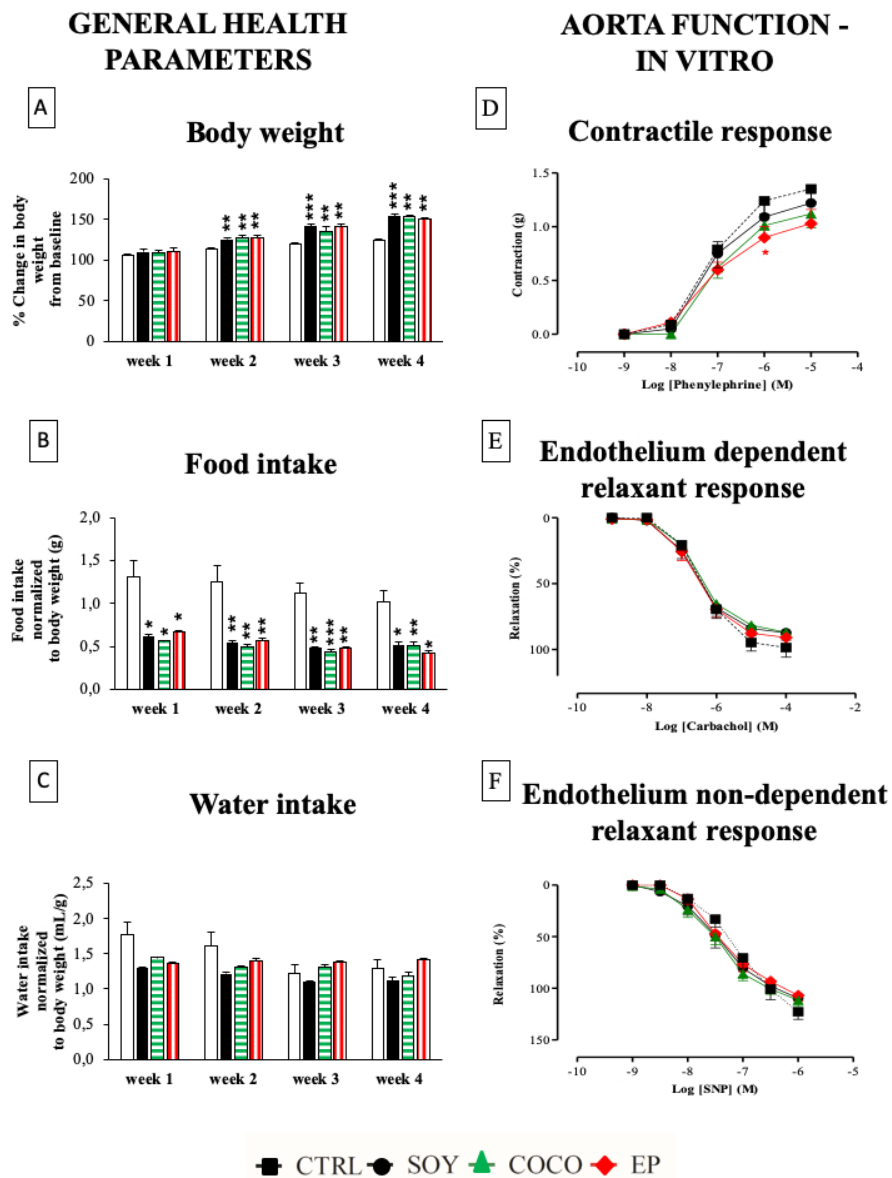
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Figure 1. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on body weight (A), food intake (B), water intake (C) and aorta function: (D) concentration-response curve of phenylephrine (10^{-9} – 10^{-5} M), (E) concentration-response curve of carbachol (10^{-9} – 10^{-4} M) and (F) concentration-response curve of sodium nitroprusside (SNP) (10^{-9} – 10^{-6} M). Results are shown as the mean ± SEM, n=6-14 rats per group. *p<0.05, **p<0.01 and ***p<0.001, vs. CTRL (two-way ANOVA followed by post-hoc Bonferroni multiple comparison test).
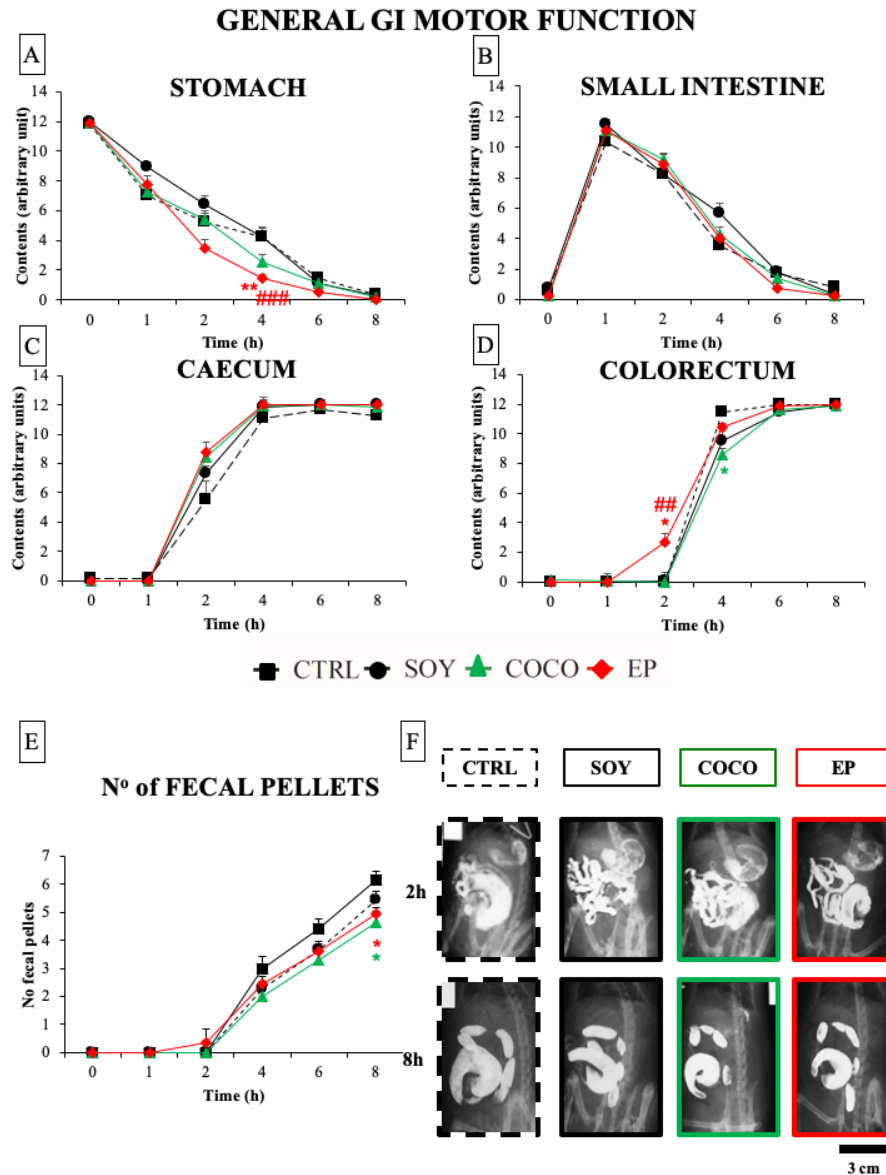
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Figure 2. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on gastrointestinal motor function and the number of fecal pellets in rats. Gastrointestinal motility was evaluated by radiological methods in: (A) stomach (gastric emptying), (B) small intestine, (C) caecum and (D) colorectum. (E) represents changes in the number of fecal pellets, whereas (F) shows representative X-rays obtained from rats, 2 and 8 h after intragastric administration of barium contrast (2.5 mL, 2 g/mL). Results are shown as the mean ±SEM (n=10-14 rats per group). *p<0.05, **p<0.01 and ***p<0.001, vs. CTRL; ##p<0.01 and ###p<0.001 vs. SOY (two-way ANOVA followed by Bonferroni post-hoc test). Scale bar: 3 cm.

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Figure 3. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on gastrointestinal motor function in rats - morphometric and densitometric studies. Morphometry and densitometry analyses of the stomach (A, B), caecum (C, D) and fecal pellets (E, F). The analysis was performed using X-ray images taken immediately and after 1, 2, 4, 6, and 8 h from time of contrast administration (Image J 1.38 for Windows, National Institute of Health, USA, free software: https://rsb.info.nih.gov/ij/). Results are shown as the mean ± SEM (n=10-14 rats per group). *p<0.05, **p<0.01 and ***p<0.001, vs. CTRL; ##p<0.01 vs. SOY (two-way ANOVA followed by Bonferroni post-hoc test).

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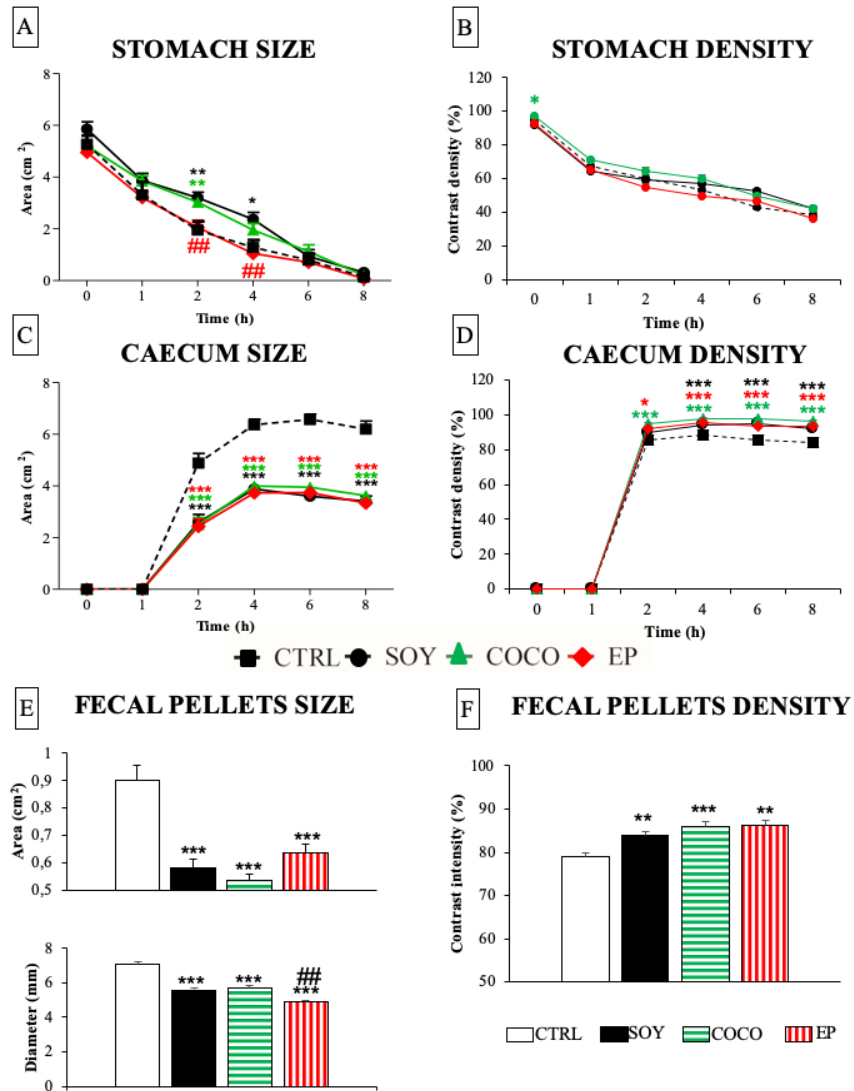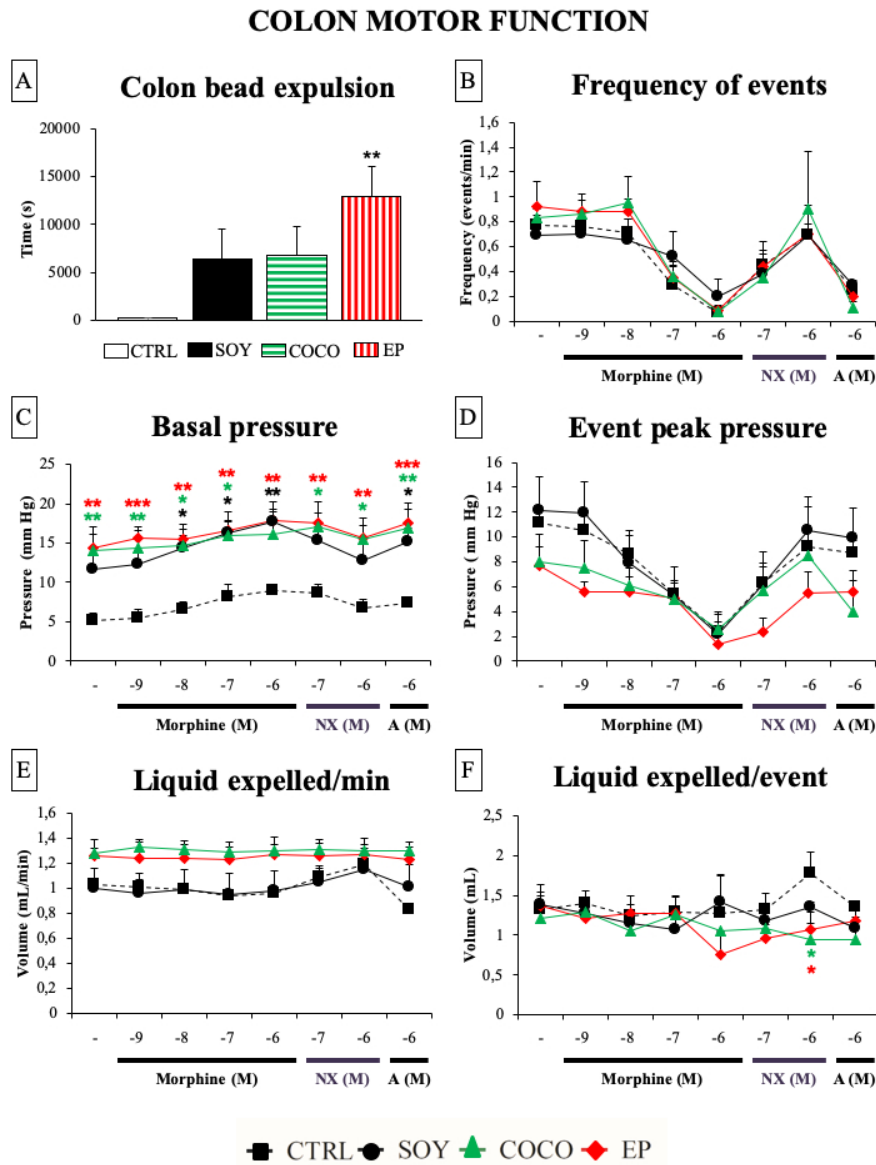
COLON MOTOR FUNCTION

Figure 4. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on (A) colonic motility assessed in colonic bead expulsion test (n= 6-7 rats/group) and (B-F) on propulsive activity of the isolated colon (n= 5-7 rats/group) . Results are shown as the mean ± SEM). *p<0.05, **p<0.01, ***p<0.001 vs. CTRL (two-way ANOVA followed by Bonferroni post-hoc test). Abbreviations: A, atropine; NX, naloxone.

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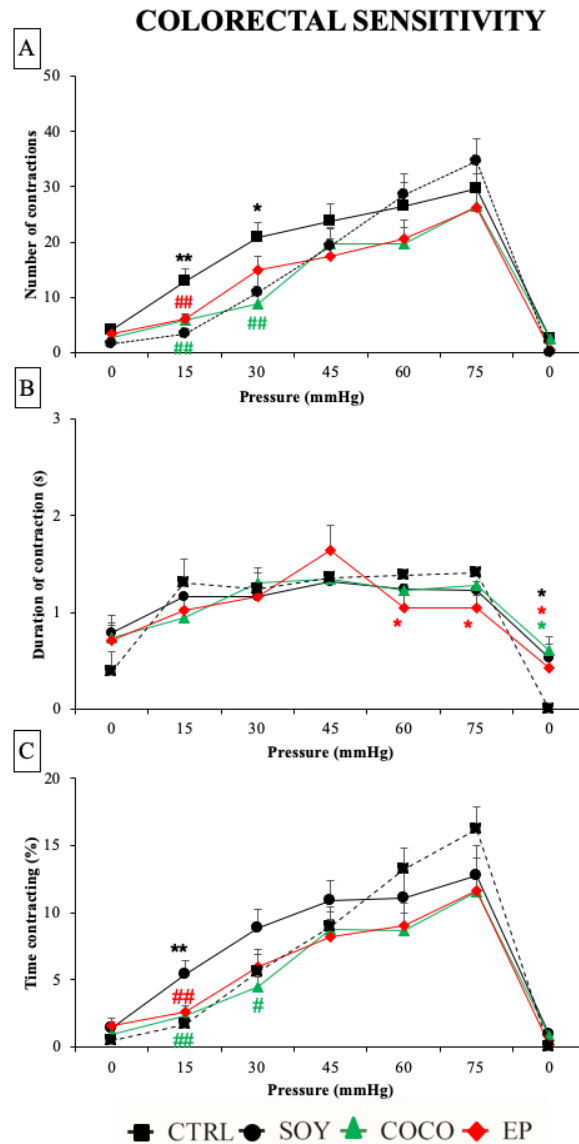51
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Figure 5. Effect of dietary treatment with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP) on abdominal contractions in response to intracolonic stimulation in rats. (A) number of abdominal contractions, (B) duration of abdominal contractions and (C) percentage of time with abdominal contractions. An intracolonic balloon was used to mechanically stimulate the colon at increasing pressures, for 5 minutes each pressure (tonic stimulation). Results are shown as the mean ± SEM (n = 6-7 animals per group). *p<0.05 and **p<0.01, vs. CTRL; #p<0.05 and ##p<0.01, vs. SOY (two-way ANOVA followed by Bonferroni post-hoc test).

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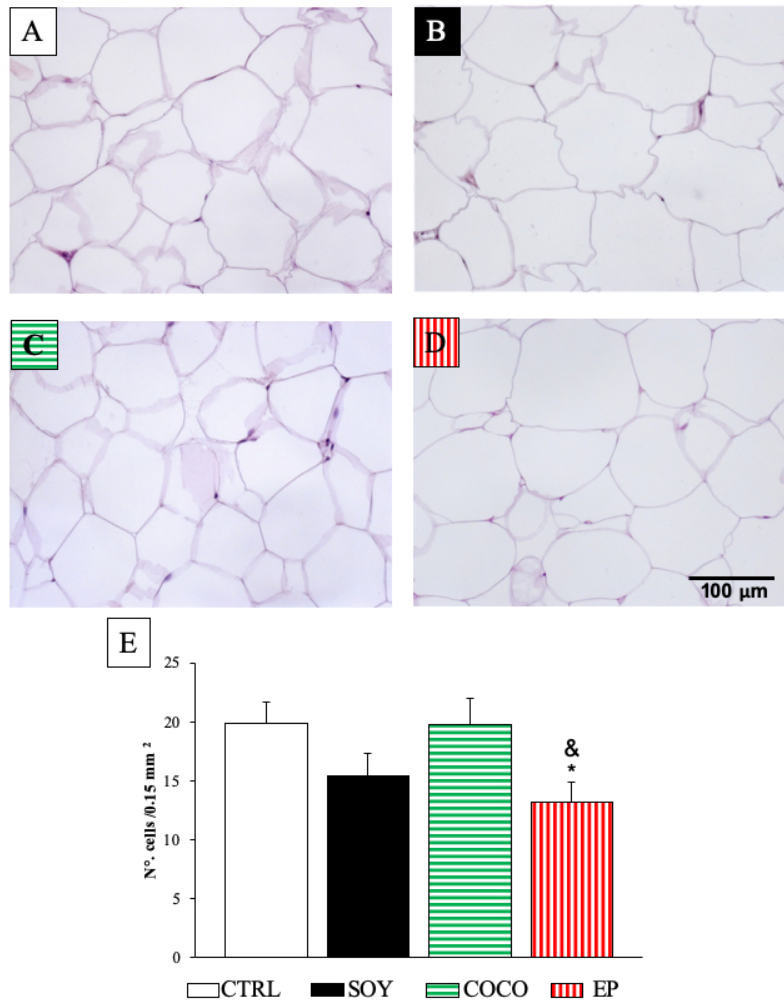
HISTOLOGY: ADIPOSE TISSUE

Figure 6. Representative images of histological samples of adipose tissue (A-D) and (E) the number of white adipocytes in the histological sections of epididymal adipose tissue (per area) of rats fed with experimental diets. Experimental groups: (A) control diet (CTRL), (B) AIN93G (SOY) diet, (C) SOY diet supplemented with coconut oil (COCO) and (D) SOY diet supplemented with evening primrose oil (EP). Results are shown as the mean ± SEM (n = 13-14 animals per group). *p<0.05 vs. CTRL; &p<0.05, vs. COCO (Student's t-test). A-D: Scale bar 100 μm.

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HISTOLOGY: MAST CELLS IN CONNECTIVE TISSUE
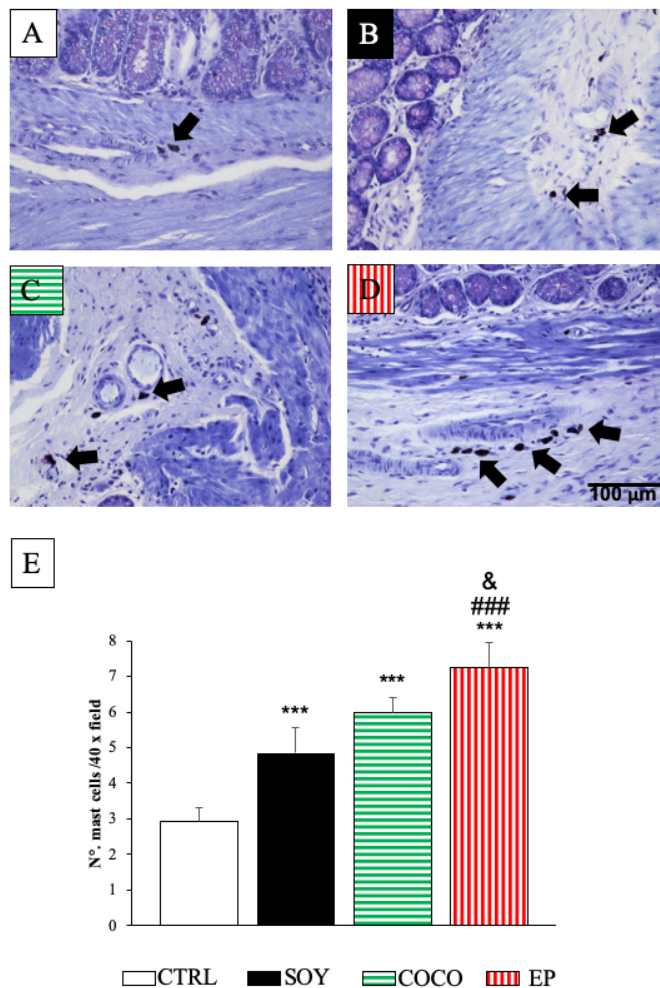
Figure 7. Effect of dietary supplementation with (A) control diet (CTRL), (B) AIN93G (SOY), (C) SOY diet supplemented with coconut oil (COCO) and (D) SOY diet supplemented with evening primrose oil (EP) on distribution and (E) average number of mast cells between the epithelium and external muscular layer (arrows) of rat colonic samples. Results are shown as the mean ± SEM (n = 4-7 animals per group) ***p<0.001 vs. CTRL; ###p<0.001 vs. SOY; &p<0.05 vs. COCO (Student's t-test). Scale bar 100 μm.

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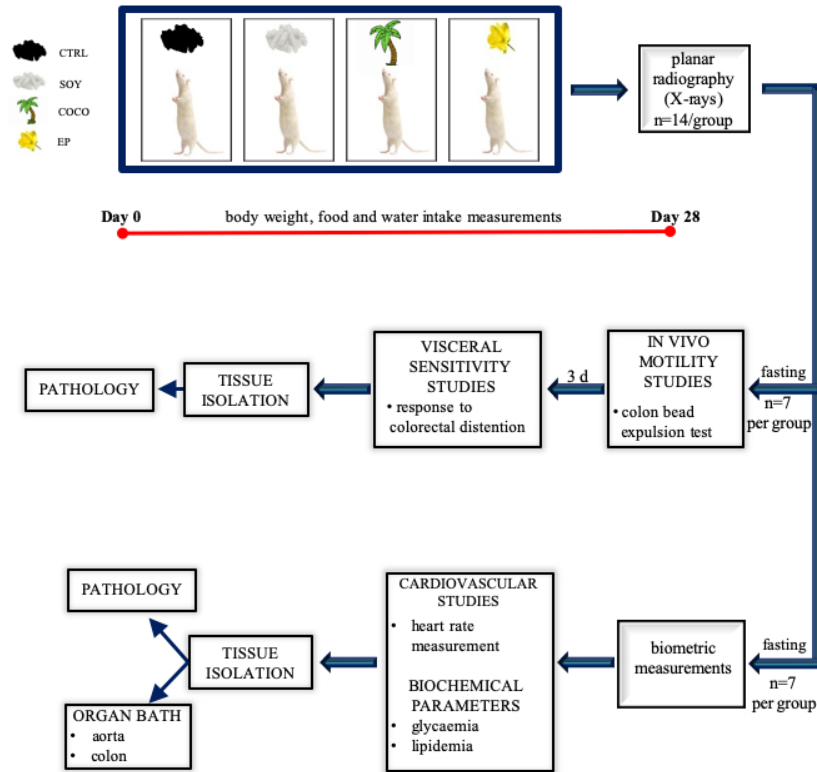22
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Figure S-1. A schematic representation of the experiments included in the study.

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Table S1. Semiquantitative analysis of the alterations in GI motility based on X-ray images. Each parameter was scored and summed for further analysis [31].

Points	Assessed parameter			
	Percentage of the GI region filled with contrast	Intensity of contrast	Homogeneity of contrast	Sharpness of the GI region profile
0	No labelling	-	-	-
0 - 1	$\leq 25\%$	Faint	Not well-defined	Heterogenous
2	$26 - 50\%$	Light	Well-defined	Homogenous
3	$51 - 75\%$	Moderate	-	-
4	$76 - 100\%$	Strong	-	-
Total score	max 4	max 4	max 2	max 2

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Table S2. Effects of dietary interventions on biometric, biochemical and cardiovascular parameters.

	CTRL (n)	SOY (n)	COCO (n)	EP (n)
Biometric parameters				
Body mass index (g/cm^2)	$0,63 \pm 0,03$ (4)	$0,66 \pm 0,03$ (6)	$0,59 \pm 0,01$ (6)	$0,65 \pm 0,02$ (7)
Lee index	$0,2940 \pm 0,0050$ (4)	$0,2950 \pm 0,0050$ (6)	$0,2850 \pm 0,0020$ (6)	$0,2950 \pm 0,0030$ (7)
Body length (cm)	$24,75 \pm 0,25$ (4)	$25,77 \pm 0,25$ (6)	$25,48 \pm 0,30$ (6)	$25,40 \pm 0,19$ (7)
Abdominal perimeter (cm)	$20,75 \pm 0,49$ (4)	$21,08 \pm 0,80$ (6)	$19,50 \pm 0,47$ (6)	$21,17 \pm 0,46$ (7)
Biochemical parameters				
Plasma glucose (mg/dL)	$60,5 \pm 6,20$ (4)	$61,86 \pm 2,03$ (7)	$66,43 \pm 2,72$ (7)	$70,14 \pm 4,19$ (7)
Plasma total cholesterol (mg/dL)	$100,0 \pm 0,0$ (4)	$101,14 \pm 1,14$ (7)	$102,14 \pm 1,39$ (7)	$100,0 \pm 0,0$ (7)
Triglycerides (mg/dL)	$269,75 \pm 32,05$ (4)	$295,29 \pm 36,76$ (7)	$384,57 \pm 36,20$ (7)	$413,57 \pm 28,52$ (7)*
Plasma HDL (mg/dL)	$49,75 \pm 8,18$ (4)	$54,86 \pm 4,96$ (7)	$53,86 \pm 7,42$ (7)	$55,43 \pm 4,72$ (7)
Atherogenic coefficient	$1,07 \pm 0,21$ (4)	$0,92 \pm 0,15$ (7)	$1,17 \pm 0,34$ (7)	$0,89 \pm 0,16$ (7)
Cardiovascular general parameters				
Systolic blood pressure (mm Hg)	$148,43 \pm 11,82$ (5)	$134,99 \pm 9,82$ (6)	$123,26 \pm 11,21$ (6)	$127,29 \pm 8,21$ (7)
Diastolic blood pressure (mm Hg)	$108,75 \pm 7,59$ (5)	$103,50 \pm 9,34$ (6)	$101,05 \pm 6,80$ (6)	$101,74 \pm 6,88$ (7)
Heart rate (beats/minute)	$329,22 \pm 18,85$ (5)	$358,18 \pm 8,92$ (6)	$350,16 \pm 13,45$ (5)	$382,10 \pm 22,15$ (7)

Values are mean ± SEM. Number of animals used is shown in parenthesis. One-way ANOVA followed by Bonferroni's post-hoc test. * $p<0.05$ vs. CTRL.
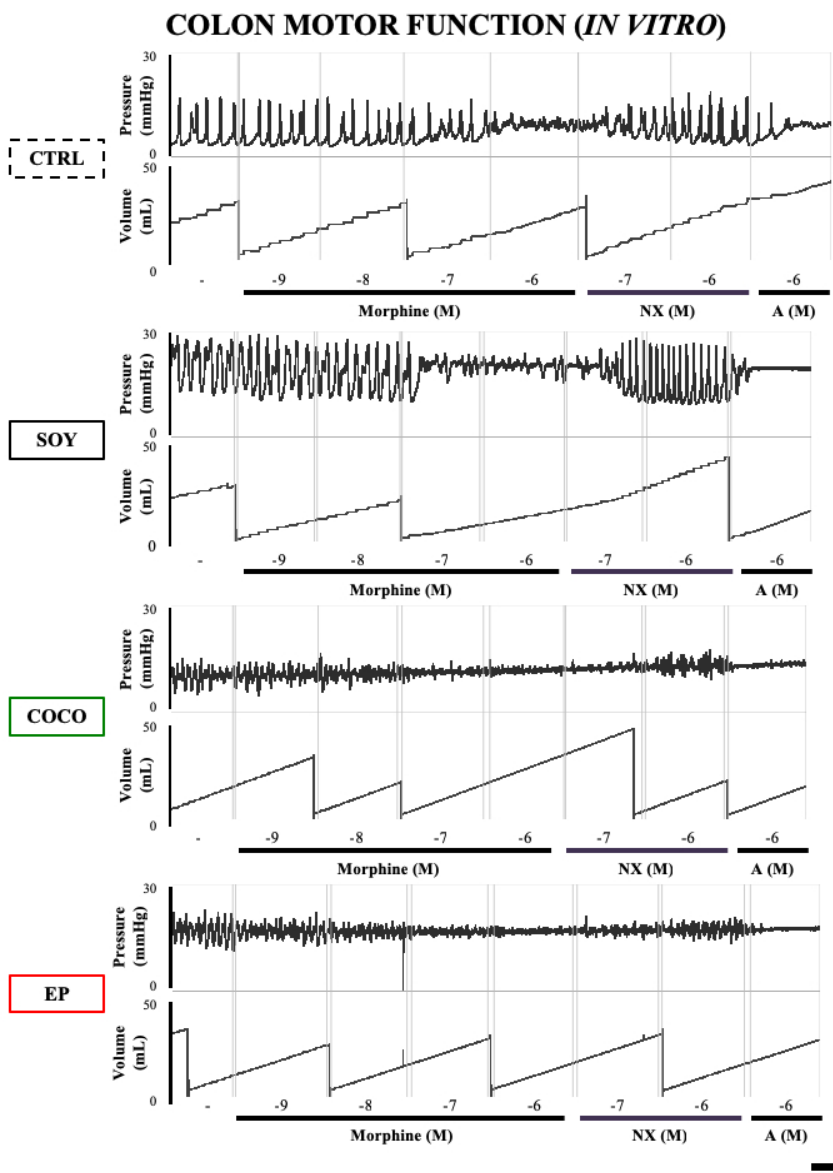
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Figure S-2. Representative recordings of the colonic propulsive activity after dietary feeding with control diet (CTRL), AIN93G (SOY), SOY diet supplemented with coconut oil (COCO) and SOY diet supplemented with evening primrose oil (EP). Abbreviations: A, atropine; NX, naloxone. Scale bar: 10 min.

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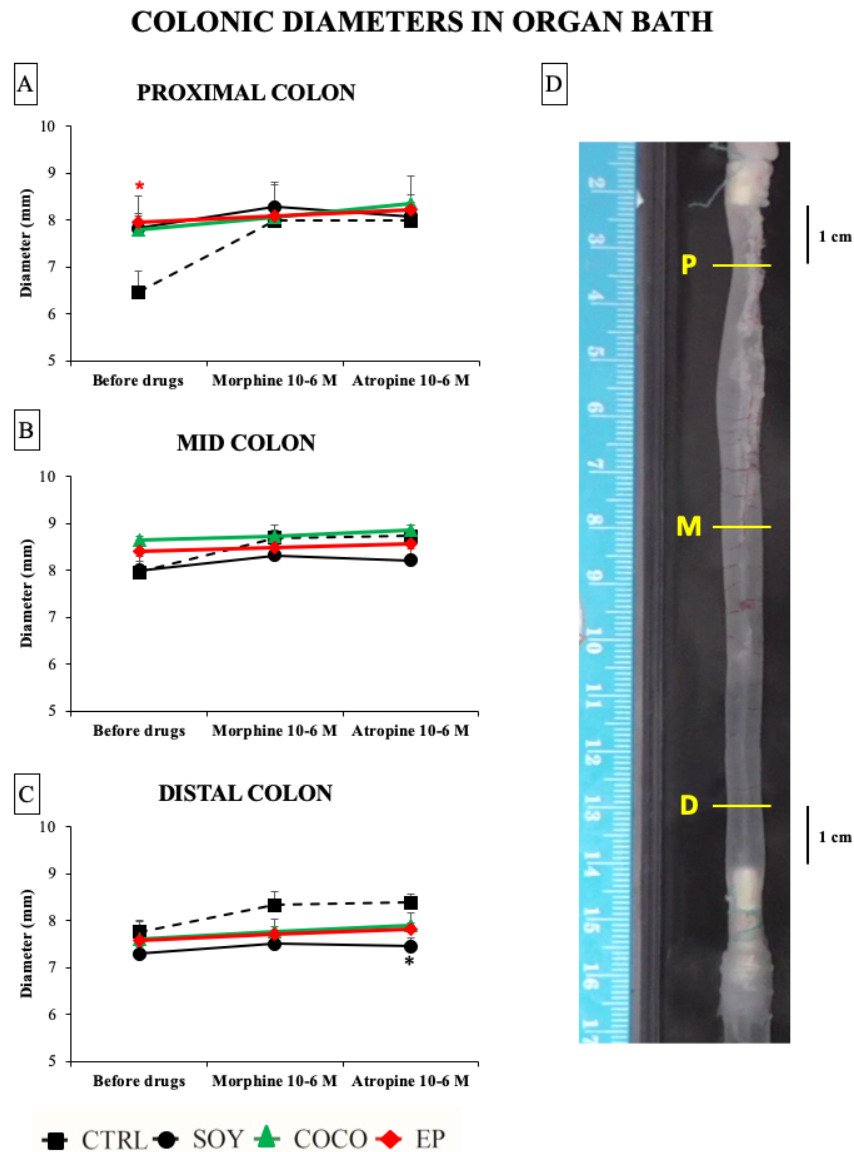
COLONIC DIAMETERS IN ORGAN BATH

Figure S-3. The effects of dietary interventions on the diameter of the proximal (A), mid (B) and distal (C) colon. (D) Representative frame from a video recorded from a CTRL-fed rat colon during organ bath experiment (see main text for details regarding organ bath experiments). Briefly, movies of colonic motor patterns were recorded with domestic camera, positioned above the preparation. Fluid was infused from the inlet located at the proximal end (top). The contents exit the colon with each peristaltic contraction through the outlet, located at the distal end (bottom). The recordings were resembled down to obtain images (frames) per second. Diameters were measured, using Image J, at three different positions: P-proximal, M-mid and D-distal (1 cm from proximal or distal end, and at the mid-point of the colon). The diameter at each point along the length of the colon was calculated for 5 different frames (at least one second apart) belonging to one animal. Frames in which persistaltic contractions were on-going, have not been taken into account. Results are shown as the mean ± SEM (n = 4-7 animals per group). *p<0.05 vs. CTRL (two-way ANOVA followed by Bonferroni post-hoc test).

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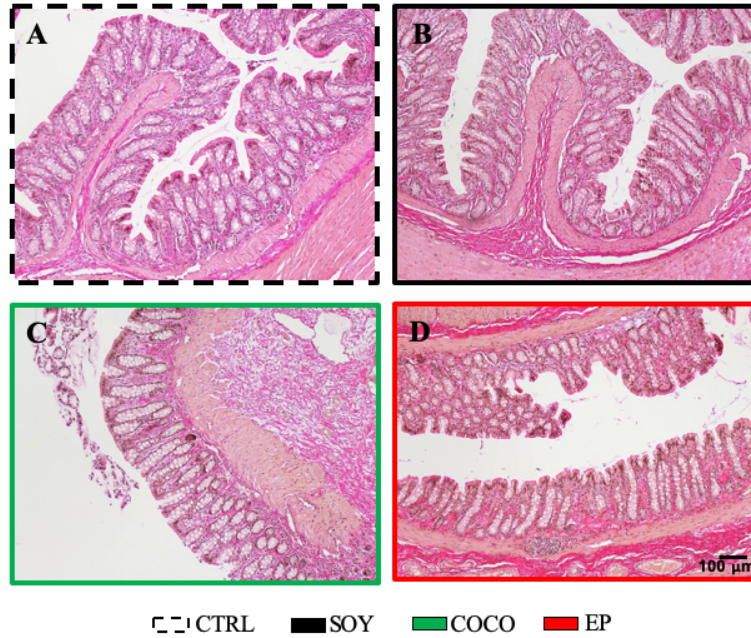51
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HISTOLOGY: COLON MORPHOLOGY

Figure S-4. Effects of dietary interventions on the general structure of the colonic wall. Animals fed with CTRL (A), SOY (B), COCO (D) and EP (E). Van Gieson´s trichrome stain. Scale bar: 100 μm.

190x254mm (96 x 96 DPI)