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EXPERIMENTAL STUDY

The influence of food restriction on the small bowel: Does intensive short-term food restriction lead to weight loss?

Makovicky P[1,2], Tumova E[3], Volek Z[4], Makovicky P[5], Arnone JM[6], Svecova I[1,2], Samasca G[7]

Czech Centre for Phenogenomics, Division BIOCEV, Vestec, Czech Republic. **pmakovicky@email.cz**

ABSTRACT

The objective of this study was to show morphological changes in the small intestine of Hyplus broiler rabbits following an eight-day food restriction programme. The control group (C) received food *ad libitum* (ADL) for the duration of the experiment. Group R1 received 50g of food per day, and group R2 received 65g of food per day. After the food restriction diet had been completed, groups R1 and R2 were returned to *ad libitum* feeding. After food restriction and at the end of the experiment, the longest small bowel measurement was recorded in the C group. In the C group, after food restriction, the villi height was significantly higher, compared to that in R1 and R2 groups and at the end of the experiment, the villi were significantly higher in R1 and R2 groups. After food restriction, the values of crypts depth were approximately similar in all groups, and the end of experiment, the depth of crypts were deepest in R1and R2 groups, as compared to that in C group. The full process is followed by weight loss to the end of the experiment. These data suggest that intensive short-term food restriction followed by ADL feeding has effect on weight loss *(Fig. 3, Ref. 30)*. Text in PDF *www.elis.sk.*

KEY WORDS: experimental medicine, food restriction, rabbit, slimming, small bowel, weight loss.

Introduction

Food restriction is a well-known method for weight loss. It is also known as one of the oldest methods of treating and preventing some diseases in humans as well as animals (1, 2, 3). For example, Claudius Galen recommended this method for overweight patients (4). Intensive development of applied medicine and science relating to the effects of food restriction has resulted in identifying numerous aspects related to food restriction, but there are still aspects that remain unclear. The small bowel has several tasks, with the main one being nutrient resorption (5, 6). Quantitative differences in small bowel resorptions can also affect other organ systems and have an important impact on health. It was found that the changes in length of the small bowel, height of villi and depth of crypts are dependent on the intensity of food restriction. It has also been shown that after food restriction with subsequent transition to *ad libitum* (ADL) feeding, there is a lengthening of the small bowel compared to the control group (7). These results were documented on limited materials and currently it is not clear how food restriction affects the entire small bowel. It is an important question, which can clarify some other open questions. Mainly it is about the integrity of the adaptation of individual anatomical parts of the small bowel to the duration and intensity of food restriction. Subsequently it is also about adaptation to the post-restriction period with ADL intake and some related changes in the small bowel morphology. Such knowledge may provide answers to the question whether intensive short-term restriction is an appropriate approach to weight loss. We hypothesize that after intensive short-term food restriction with subsequent ADL feeding, the height of villi is going to grow intensively, thus increase the intestinal absorptive area and result in excessive weight gain. These findings can be very important for clinical praxis because they are related to the general use of food restriction for weight loss. It is not clear which anatomical part of the small bowel is more sensitive to food restriction and how they subsequently react to the resumption of ADL intake following food restriction. These questions can be important for physiological research and they can also find utility in studies dealing with gastrointestinal resorption. The objective of our work is to perform histological analyses of the small bowel under intensive short-term food restriction, including the degree of variability in the percentage of food restriction in combination with ADL intake in an experimental rabbit model.

[1]Czech Centre for Phenogenomics, Division BIOCEV, Vestec, Czech Republic, [2]Department of Transgenic Models of Diseases, Institute of Molecular Genetics, AS CR v.v.i., Prague, Czech Republic, [3]Department of Animal Husbandry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Czech Republic, [4]Physiology of Nutrition and Quality of Animal Product, Institute of Animal Science in Prague–Uhrineves, Czech Republic, [5]Department of Biology, Faculty of Education, Selye Janos University, Komarno, Slovak Republic, [6]School of Nursing, Kean University, Union New Jersey, U.S., and [7]Department of Immunology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj–Napoca, Romania

Address for correspondence: P. Makovicky, Ing, PhD, Czech Centre for Phenogenomics, Institute of Molecular Genetics AS CR v.v.i., Division BIOCEV, Prumyslova 595, CZ-252 50 Vestec, Czech Republic. Phone: +420776842684

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Material and methods

Animal ethics.

This study was approved by the Ethics Committee of the Institute of Animal Science and the Central Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic and was carried out in accordance with the guidelines for applied nutrition experiments in rabbits (8).

Study design

Small bowel samples from broiler rabbits kept in special experimental conditions were analysed. The experiment with Hyplus broiler rabbits was conducted in the rabbit building of the Institute of Animal Science in Uhrineves in Czech Republic from a weaning age of 32 days up to 70 days of age. All rabbits were kept under controlled environmental conditions and housed in standard cages. They were divided into three groups. The control group was fed ADL during the entire experiment (C). The first experimental group was restricted between 35 and 42 days of age, when the rabbits received 50 g of food per rabbit per day (R1). The second experimental group was also restricted between 35 and 42 days of age, but the rabbits obtained 65 g per rabbit per day (R2). Before and after restriction, all rabbits were fed ADL with a commercial food mixture. The design of the experiment is shown in Figure 1.

Sample collection

For small bowel analysis, eight rabbits per group were weighed and then slaughtered at 42, 49 and 70 days of age, from all three (C, R1, R2) groups. The length of the entire small intestine, from pylorus to ileocaecal junction, was measured and then three tissue samples were obtained from each rabbit, namely from the duodenum, jejunum and ileum. All samples were collected within 30 minutes after slaughtering and from the same anatomical place. Small bowel samples were fixed with a 4 % Bouin solution for two days.

Sample evaluation and processing

Samples were processed by standard histological methods and then embedded in paraffin blocks. The slices were stained with haematoxylin-eosin (DiaPath, Italy). The height of the villi and the depth of the crypts were measured using the NIS-Elements AR version 3.0 software (Laboratory Imaging s.r.o., Czech Republic). The height of villi was measured from the top of the villus to the crypt transition, and the crypt depth was defined and measured as the invagination between two villi. The heights of 50 villi, and the depths of 50 crypts were individually measured per each duodenum, jejunum and ileum sample.

Statistical analysis

The results were evaluated using the SAS program (9), and the ANOVA method ($p < 0.05$).

Results

The results of weight, length and measurements of villi heights and crypt depths are shown in Figure 2. All morphometric measurements, segregated according to the different parts of the small bowel, are shown in Figure 3.

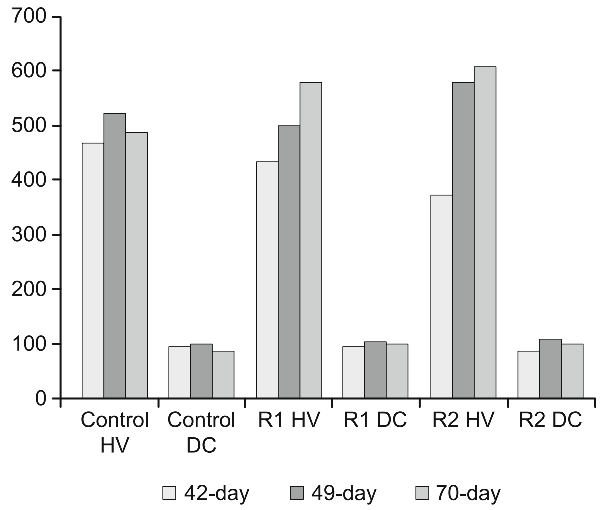

Fig. 1. The height of villi and depth of crypt in control, R1 and R2 groups. R1 – restriction 50 g of food per rabbit/per day, R2 – restriction 65 g of food per rabbit/per day, HV – height of villi, DC – depth of crypts.

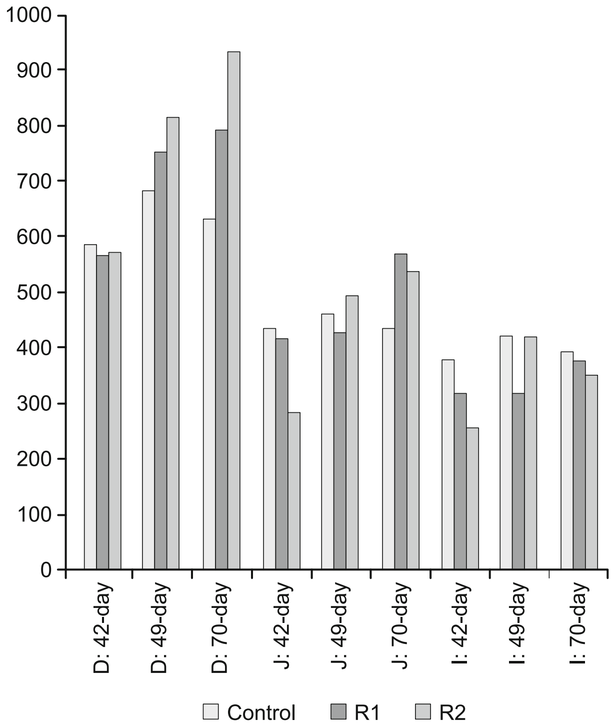

Fig. 2. The height of villi in different part of small bowel. R1 – restriction 50 g of food per rabbit/per day, R2 – restriction 65 g of food per rabbit/per day, D – duodenum, J – jejunum, I – ileum.

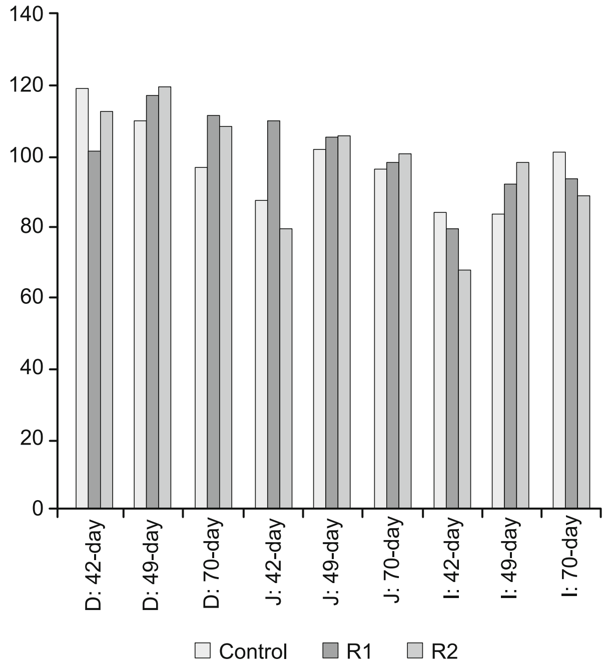

Fig. 3. The depth of crypt in different part of the small bowel. R1 – restriction 50 g of food per rabbit/per day, R2 – restriction 65 g of food per rabbit/per day, D – duodenum, J – jejunum, I – ileum.

Weight of rabbits

After restriction, there were some differences between rabbits' weights, with the heaviest average weight measured in the C group. Almost continuous weight gain occurred in all groups with the maximum weight recorded at the end of experiment for all groups when some differences were visible ($p < 0.05$).

Length of the small bowel

Immediately after restriction at the first sampling the longest small bowel measurement was recorded in the C group. There was a further increase in the length of the small bowel observed to the end of the experiment.

Villi height

Results show changes between C group and R1, R2 groups in some measured parameters. In the C group, immediately after food restriction on day 42, the villi were significantly higher, compared to R1 and R2 groups ($p < 0.05$). At the end of the experiment, the situation was opposite, with the villi being significantly higher in R1 and R2 groups ($p < 0.05$). As to the different anatomical parts of the small bowel, immediately after food restriction, a minimal change in duodenal villi height was visible among all three groups. At the end of the experiment, the R1 and R2 groups had a higher duodenal villi height, compared to the C group ($p < 0.05$). Immediately after food restriction, jejunum villi were higher in the C group ($p < 0.05$). At the end of the experiment the situation changed. The jejunum villi were highest in R1 and R2 groups ($p < 0.05$). The morphometric analysis of ileum villi height shows that in all three groups there was gradual villi growth visible, but the ileum villi were highest in the C group from the start to the end of the experiment.

Crypt depth

The results show changes between C group and R1, R2 groups in the measured parameters. Immediately after food restriction, the values of crypts depth were approximately similar in all groups, but at the end of experiment, the depth of crypts were deepest in R1 and R2 groups, as compared to the C group. The crypt deepening was observed in all three groups after ADL feeding, but at the end of experiment the value was lower compared to ADL feeding immediately after food restriction. At the beginning of the experiment, the duodenal crypts were deeper in the C group, compared to the R1, R2 groups, but at the end of the experiment the situation reversed and the duodenal crypts were deeper in R1and R2 groups. The crypt depth in the jejunum exhibited some changes after food restriction, while at the end of experiment, R1 and R2 group crypts were deeper. From the beginning, and at the end of experiment, the ileum crypts were deeper in the C group, compared to R1 and R2 groups, with minimal differences immediately after ADL feeding in R1 and R2 groups.

Discussion

Today it is known that variability in diet can modify small bowel morphology, including the height of villi and depth of crypts (10). There is plenty of information in literature about the effects of food restriction on the small bowel. The results of an experiment in rats subjected to a 50 % dietary food restriction showed that there was hypertrophy of the duodenal mucosa and atrophy of the ileal mucosa (11). The numbers of goblet cells and intraepithelial lymphocytes were reduced, but the mitotic index remained unaltered in both the duodenum and ileum. In our experiment with rabbits, we do not find the same histopathological changes in the small bowel. The observed changes in small bowel morphology suggest that intensive short-term food restriction can be beneficial for subsequent, more efficient resorption. This effect is even observed in less intensive food restriction (65 g/per rabbit), and not just in the more stringent food restriction (50g/per rabbit). Other groups' findings are in concordance with these observations and have been ascribed to gastrointestinal immunity modulation and gut microbiota (12, 13). The work of Tsiouris et al shows that feed restriction of broiler chicks has a positive effect on the intestinal ecosystem and significant protective effect against necrotic enteritis in a subclinical experimental model (14). The results of Knudsen et al show that feed-restricted rabbits tended to have greater expressions of interleukin and lower expressions of tumour necrosis factor-α, suggesting that food restriction can modulate gut immunity (15). It has been also shown, that small bowel morphology changes during fasting and refeeding are accompanied by heat shock protein modulation (16). We assume, that there are some influences and dependencies between individual parts of small bowel and food restriction. The duodenal part was minimally affected by intensive short-term food restriction, but on the other hand, it is the most sensitive part to ADL intake. The jejunum show a less sensitive reaction in the post-restriction period with ADL intake than the duodenum. The ileum part is least affected by the post-restriction period with ADL intake. These results indicate that the duodenum is less affected by short-term food restriction, and more affected by subsequent ADL feeding, while in the distal direction, the effect of intensive short-term food restriction becomes stronger, but the reaction to subsequent ADL feeding is less intense. So altogether it seems that the proximal part of the small bowel can absorb the most nutrients from the restricted food intake, just as well as from ADL food intake. The results of Tumova et al shows that a one-week feed restriction can modify the morphology of intestinal tract of a growing rabbit (17). At the end of this experiment, the dietary-restricted rabbit group had the longest small bowel, highest villi and deepest crypts, compared to ADL-fed rabbits. Our work has deepened these findings, as we further analysed the different parts of the small bowel. Our results show that short-term food restriction has a potential beneficial effect for health, because there is no decrease in villi height that has been generally associated with reduced intestinal health. In the experiment of De Oliveira et al, dietary restrictions from 33 to 40 days of age and from 54 to 61 days of age, were associated with no effects on duodenal morphometry (18). In the jejunum, the villi of ADL-fed rabbits or of those with diet restricted from 33 to 40 days were higher, and rabbits with diet restricted from 33 to 40 days had wider villi and higher absorption surface. In the ileum, rabbits fed ADL had higher villi. The work also showed that feed restriction reduced the heart weight, but not its size, and negatively affected jejunum morphometry when performed in later stages. At the same time, literature contains conflicting data indicating that feed restriction does not significantly influence performance indices, carcass yield, organ weights and body dimensions (19). Another work shows that in addition to decreasing the enzymatic activity in the enterocytes, the feed restriction can result in lower villus perimeter and lower crypt depth, (20). In our previous work, we have shown that feed restriction in rabbits can affect small bowel morphology in the duodenum (21). In our first work dealing with food restriction applied on rabbits between 42 and 49 days, significant interactions ($p < 0.05$) between group and age were documented as to villi height and crypt depths of duodenum (22). These findings support the theory that villus height and crypt depth and length of the small intestine change with the intensity of feed restriction and age. Considering intensive short-term food restriction and weight loss, there is a gap in current knowledge. It is assumed that dietary food restriction prevents excessive body weight gain (23). For a long time, researchers have searched for appropriate overweight and obesity treating tools. Long-term food restriction looks like one possibility to reduce weight. Restricted feeding, and thus limited caloric intake, prevents many health problems and increases animal lifespan (24). It is also a recommended method in human clinical practice. Nevertheless, a question should be posed as to whether intensive short-term food restriction followed by ADL feeding is a really good method for weight loss. In our experiments, we have demonstrated that immediately after intensive short-term food restriction, followed by ADL feeding, there were increases in the length of villi in the small bowel. Our results documented that immediately after food restriction, the height of villi in R1and R2 groups were smaller, compared to the C group. After a short ADL feeding period, there was an intensive growth period and the villi were highest in R1 and R2 groups, compared to the C group. This intensive growth was subsequently reflected with an increase in absorption surface, thus leading to a better use of nutrients by the body. Immediately after food restriction, the weight of rabbits in R1 and R2 groups were lower, compared to the C group, while the same results were observed at the end of experiment. Full period was accompanied by increasing height differences. At 42 days of age there were some weight differences between C and R1 (–43 g); C and R2 (–32 g). At 49 days of age the differences were greater, namely between C and R1 (–131 g); C and R2 (–40 g) while at the end of experiment they were as follows: C and R1 (–343 g); C and R2 (–380 g). The results showed, that an intensive short-term food restriction is an appropriate instrument for gastrointestinal tract recovery and has effects on weight loss. Other references showed, that a more effective instrument for weight loss is the intermittent fasting or alternate day fasting. It is clear, that it is not a universal medicine and also that one approach does not fit all in the quest for achieving body weight control, but this dietary strategy could be considered as an option for achieving weight loss and its maintenance (25). The findings of Zhang et al show that simply reducing the amount of food intake results in an increased appetite accompanied with obvious weight regain, and suggested that the resulting enlargement of villi surface area plays a key role in the regain of weight (26). The experimental work of Dou et al described an intensive 7-day fasting on a rat model and showed that the rats regained the lost body weight (22 %) by the 7th day of re-feeding (27). The lost duodenal mass (40 %) and jejunum mass (25 %) were regained by the 2nd day whereas the lost mass from the ileum (18 %) was regained by the 4th day. The fasting-induced morphometric changes were normalised by re-feeding on the 2nd day in the duodenum and jejunum, and on the 4th day in the ileum. The results of another important work dealing with the influences of short 2-day fasting periods, show significantly decreased iron concentrations in serum and hair, as well as levels of ferritin, haemoglobin, haematocrit, red blood cells, and total iron binding capacity, however this short-term fasting did not influence the other iron management parameters (28). These changes can alter the emotional status of slimming women and can be in association with depression. This may be a global worldwide problem because many women are discontented with their appearance, including weight (29, 30). They indicate that some restrictive diets can be finally harmful, but most of them still stay ineffective following weight gain. The results of our work explain that weight loss based on intensive short-term food restriction, when followed by ADL feeding, is compensated by improved nutrient resorption due to the expansion of small bowel resorption capacity, and has effects on weight loss.

Conclusion

This work confirms, that an intensive short-term food restriction influences all three parts of the small bowel. Intensive short-term food restriction followed by ADL feeding has effects on weight loss.

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