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Effect of fasting in the digestive system: Histological study of the small intestine in house sparrows

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ABSTRACT

In birds and mammals the metabolic response to fasting has been studied and can be characterized by three consecutive phases reflecting metabolic and physiological adjustments. An effective way to minimize energy expenditure during food scarcity is to decrease the mass of the organs. As the digestive system is metabolically expensive to maintain, the small intestine and the liver are the most affected organs. We evaluated the effects of phase III starvation on the mass of the different organs and histological parameters on house sparrows, a small non-migrant bird. In a short period of time (34 h) we observed a larger reduction in the digestive organ mass when compared to the mass of the body and non-alimentary tissues. Furthermore, the intestinal mass was proportionally more reduced than its length and nominal surface area. A reduction on the intestinal mucosal layer also resulted in a shortening of villus (length and thickness) and crypt depth. Moreover, the morphology of the enterocytes changed from cylindrical to cubical, suggesting that the surface exposed to the lumen was conserved. This may indicate an adaptive response to the moment of refeeding. The nominal surface area/body mass remained constant in both groups and several histological parameters were reduced, suggesting that starving induces the atrophy of the small intestine. However, the goblet cells were conserved after fasting indicating a protective tendency.

1. Introduction

In birds and mammals the effects of fasting can be described by three consecutive phases. These phases can be defined by progressive metabolic and physiological changes (Chediack et al., 2012; McCue, 2010; Wang et al., 2006). Phase I occurs immediately after the last food has been absorbed by the small intestine. It is characterized by the use of the liver glycogen stores and a significant reduction in body mass in a short period of time. Phase II is characterized by energy preservation and lipid oxidation. Since lipids have a high amount of energy, weight loss is slow during this stage. Finally, during phase III, when lipid deposits are almost depleted, muscle protein catabolism begins combined with a decrease in protein synthesis. During this phase, there is a remarkable reduction in body and organ mass (Wang et al., 2006) resulting from starvation combined with the energy demands required to maintain the body homeostasis (Bauchinger et al., 2005). Thus, an effective way to minimize energy expenditure during food scarcity is to decrease the mass of certain organs.

The digestive system is metabolically expensive to maintain, the regulation of its physiological functions requires about 17–25% of the whole body oxygen consumption (Cant et al., 1996). It has been found that the small intestine and the liver are the most affected organs during fasting (Chediack et al., 2012; Ferraris and Carey, 2000; Starck, 2003). Throughout starvation animals display morphological changes in the gastrointestinal tract. These include a decrease in the length and mass of the small intestine, a decrease in mucosal weight, changes in villus length and thickness, and phenotypic changes in the enterocytes (Dunel-Erb et al., 2001; Karasov et al., 2004; Zeng et al., 2012). In rats and birds the generative components (crypts) of the mucosa are preferentially preserved in

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comparison with the absorptive part (villi) during fasting (Dunel-Erb et al., 2001; Karasov et al., 2004). The effects of fasting on the muscular layer are important in rats (Dou et al., 2002), while absent in fish and passerine wild birds (Karasov et al., 2004; Zeng et al., 2012). Several factors including body mass affect the rate at which starving animals (birds and mammals) lose body mass, making it highly variable. On a comparative level, mammals and birds with similar body mass, such as sparrows and mice (~25 g each), have a notable difference in their fasting tolerance times, 3–4 days for the mouse (Sokolovic et al., 2007; Wang et al., 2006) and 31–36 h for the sparrow (Chediack et al., 2012; Khalilieh et al., 2012). This disparity between mammals and birds may be explained by the higher basal metabolic rate of the latter, between 30 and 40% higher than that of mammals (McNab, 2009). In mice there are controversial observations about the significance of the structural changes observed during starvation, mainly in the third phase, where some researchers found varied histological changes while others did not (Chappell et al., 2003; Nian et al., 2002; Shin et al., 2005; Sokolovic et al., 2007). In birds, observations in a migrant passerine wild bird (*Sylvia atricapilla*) showed a significant effect of fasting in the reduction of the structure of the gastrointestinal tract (Karasov et al., 2004). Despite previous studies in house sparrows reports changes in organ morphology during fasting (Chediack et al., 2012), this is the first exhaustive analysis of intestinal histology changes on a non-migrant adult passerine bird undergoing fasting.

Our objectives were to evaluate the effects of long-term starvation on organ mass loss, and show how starvation affects the histology of the small intestine (perimeter, muscular layer, mucosal layer, villus length, crypt size, enterocyte morphology and goblet cell).

2. Materials and methods

2.1. Animal care and housing

Adult house sparrows (*Passer domesticus*) were captured with a live trap near the Universidad Nacional de San Luis campus (San Luis, Argentina). The birds were housed in cages (40 cm × 25 cm × 25 cm) indoors under relatively constant environmental conditions (23 ± 1 °C and 40 ± 10% of relative humidity) on a photoperiod of 14:10 h (light:dark) with food and water ad libitum (seeds supplied with vitamins and minerals). Animals were acclimatized to laboratory conditions for at least two weeks prior experimentation. Animal care and trial protocols (protocol number N° B69/09) were approved by the committee of Universidad Nacional de San Luis (CICUA).

2.2. Experimental design

2.2.1. Experiment: Effect of long-term fasting on organ mass and intestinal morphometric parameters.

Adult house sparrows ($n = 22$) were randomly assigned to either the feeding or long-term fasting group with water ad libitum ($n = 11$ for each experimental group). The mean body mass of both groups was similar at the beginning of the experiment (25.66 ± 0.88; 25.56 ± 0.40, $p > 0.92$). On the fasted group, food was removed 2 h after lights turned on (8:00 h). 24 h later the bird's body mass was measured every 2–3 h until phase III of fasting, when animals lost more than 15% of their body mass (~31–34 h). At the end of the experiment, birds were anesthetized using ketamine and xylazine (Paul-Murphy and Fialkowski, 2001), the abdominal cavity opened and the entire gastrointestinal tract removed and chilled in ice-cold avian saline buffer. The small intestine, stomach, pancreas, heart and liver were removed, cleaned of extraneous tissue and weighed. The small intestine lumen was washed with ice-cold avian saline buffer to remove digesta and measured. For histological procedures four animals were used in each group; the remaining animals were used for another experiment.

2.2.2. Enterocytes isolation

Enterocytes were isolated using a modification of a classical chemical method adjusted for birds (MacDonal et al., 2008). Briefly, small intestinal segments were washed with ice-cold Hanks' balanced salt solution supplemented with mannitol (HBSS–mannitol, pH 7.2). Intestinal segments were submerged in 1 mL of ice-cold chelation buffer containing: 30 mM EDTA, 1.94 mM DL-dithiothreitol, 52 mM NaCl, 4.39 mM KCl, 10 mM L-1N-2-hydroxyethylpiperazine-N′-2-ethanosulfonic acid (HEPES), 60 mL/L HCl 1 N (pH 7.1) for 75 min at 4 °C without shaking. After that the chelation buffer was gently discarded and 1 mL of HBSS–mannitol added and cells vigorously shaken for 30 s. Cells were collected and centrifuged at 100 × *g* during 5 min at 4 °C. The pellet was washed three times by successive resuspension in 1 mL of HBSS–mannitol. The cells were dispersed by passing them several times through a hypodermic needle and centrifuged at 100 × *g* for 5 min at 4 °C. The protein concentrations of the enterocyte samples in the three intestinal portions were estimated using the Bradford protocol.

2.3. Analytical procedures

2.3.1. Histological procedure

Proximal, medial and distal intestinal portions were placed in individual vials containing fresh Bouin solution (saturated solution of picric acid, with glacial acetic acid and formol 40%) and allowed to fix for 6–12 h at room temperature, for posterior processing. The intestinal pieces were dehydrated in increasing ethanol concentrations, cleared in xylene, embedded in paraffin and 5-μm thick sections were obtained with a microtome (Microm HM 325). Sections were mounted on slides, stained with hematoxylin-eosin or Periodic acid-Schiff (PAS) methods and covered with cover glasses.

A computer-assisted image analysis system was used to measure the percentage of area of PAS-positive vesicles and the number of cells per reference area, circumference of the serosal surface, length and width of villi, width of the crypts, length and width of enterocytes. The system consisted of an Olympus BX-40 binocular microscope interfaced with a host computer, image processing and recording system. The images were captured by a Sony SSC-DC5OA camera (Sony Corp., Tokyo, Japan) and processed with Image-Pro Plus 5.0 software (Media Cybernetics Inc., Bethesda, MD, USA) under control of a Pentium IV computer. The software allowed the following processes: image acquisition, automatic analogous adjustment, thresholding, background subtraction, distance calibration, area and diameter measuring, and disk data logging. The image was displayed on a color monitor, and the parameters were measured with the image analysis system. Before counting, a standard area of 18,200 μm^2 (reference area, RA) was defined on the screen, and distance calibration was done using a slide with a micrometric scale for microscopy (Reichert, Austria).

- Percentage of area of PAS-positive vesicles (% AV) was calculated using the formula % AV = $\Sigma Av/\Sigma RA \times 100$, where ΣAv is the sum of the area of PAS-positive vesicles and ΣRA is the sum of the small intestine area of every microscopic field.
- The number of goblet cells (No. cell/RA) with a visible nucleus was counted in 10 microscopic fields per section. The result was expressed as number of cells per RA.
- In each section the circumference of the serosal surface, length and width of villi, width of the crypts and length and width of enterocytes was measured. At least 30 measurements per section were taken, resulting in more than 90 measurements per