Effects of Physical Exercise on the Intestinal Mucosa of Rats Submitted to a Hypothalamic Obesity Condition

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ABSTRACT

The small intestine plays a role in obesity as well as in satiation. However, the effect of physical exercise on the morphology and function of the small intestine during obesity has not been reported to date. This study aimed to evaluate the effects of physical exercise on morphological aspects of the rat small intestine during hypothalamic monosodium glutamate (MSG)-induced obesity. The rats were divided into four groups: Sedentary (S), Monosodium Glutamate (MSG), Exercised (E), and Exercised Monosodium Glutamate (EMSG). The MSG and EMSG groups received a daily injection of monosodium glutamate (4 g/kg) during the 5 first days after birth. The S and E groups were considered as control groups and received injections of saline. At weaning, at 21 days after birth, the EMSG and E groups were submitted to swimming practice 3 times a week until the 90th day, when all groups were sacrificed and the parameters studied recorded. Exercise significantly reduced fat deposits and the Lee Index in MSG-treated animals, and also reduced the thickness of the intestinal wall, the number of goblet cells and intestinal alkaline phosphatase activity. However, physical activity alone increased the thickness and height of villi, and the depth of the crypts. In conclusion, regular physical exercise may alter the morphology or/and functions of the small intestine, reducing the prejudicial effects of hypothalamic obesity. Anat Rec, 299:1389–1396, 2016.

Key words: animal models; exercise; obesity; MSG; small intestine

INTRODUCTION

Obesity is a multifactorial disease and the World Health Organization estimated that, by 2015, ~2.3 billion adults would be overweight and more than 700 million would be obese (Suzuki et al., 2010). The great quantities of food consumed during growth (over nutrition) may also result in obesity in adulthood, resulting in an increase in body fat storage (Von Diemen et al., 2006).). In contrast to our ancestors, who walked a lot of miles to find food, modern human life is characterized

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by sedentary behavior coupled with the availability of fast food, which are also important risk factors for the development of obesity (Armstrong et al., 2003; Kain et al., 2003; Stubbs et al., 2004; Kalies et al., 2005).

This extra fat accumulation leads to serious hypothalamic neuroendocrine disorders such as insulin resistance, as observed in the metabolic syndrome of the type 2 diabetes (T2D), nonalcoholic fatty liver disease, non-insulin-dependent diabetes mellitus, heart diseases such as atherosclerosis and stroke, and certain types of cancer (Suzuki et al., 2010) . Therefore, obesity is widely studied in humans and also in animal models using different approaches such as genetic changes, dietary manipulations or neuroendocrine disruption (Choi et al., 1999; Von Diemen et al, 2006).

In rats, one strategy to create several neuroendocrine changes in adulthood that are similar to those found in obesity, is to induce the apoptosis of the neurons present in the arcuate nucleus of the hypothalamus, which regulates the satiety process (Menendez et al., 1990, Mercer et al., 1996, Cone et al., 2001) using progressive subcutaneous injections of monosodium glutamate (MSG) during the first days of life (Bunyan et al 1976; Leibowitz et al., 1981; Dawson et al., 1997; Bueno et al., 2005); this method is therefore widely used in studies on obesity.

In rat, MSG also alters the production of growth hormone by the hypothalamic nucleus, reduces the development of different organs, such as the heart, lungs, spleen, pancreas, kidneys, testes, brain and submandibular glands, and increases the size of the small intestine (Hamaoka and Kusunoki, 1986). Additionally, the MSG-obese condition may affect the enzymes present in intestinal epithelial cells, such as the intestinal alkaline phosphatase (IAP). IAP is a localized brush border enzyme, related to the transport of long-chain fatty acids (Takase and Goda, 1990; Bernard et al., 1992), that has been reported to be associated with the rhythm of food intake, under normal conditions (Ishikawa et al., 1983; Alpers et al., 1995; Zhang et al., 1996). Different models for the study of obesity, however, have demonstrated that IAP is also involved in detoxification of the lipopolysaccharide (LPS) (Koyama et al., 2002; Bates et al., 2007; Geddes and Philpott, 2008) produced by the intestinal microbiota. In the MSG-obese rat model, where the animals develop a hypophagic condition, IAP activity has been found to be increased (Martinková et al., 2000), while in animals with a hyperphagic condition, IAP activity is decreased (de La Serre et al., 2010). These different results for IAP demonstrate that others factors, such as the circadian rhythm and different experimental approaches may be involved in its expression regulation.

On the other hand, regular physical exercise may change physiological parameters involved in the MSG obesity condition, such as glucose and lipid homeostasis (Scomparin et al., 2006; Andreazzi et al., 2009; Scomparin et al., 2009; Scomparin et al., 2011) . However, little knowledge is available regarding the effects of physical exercise on the gastrointestinal morphology of the MSG-obese rat model. As the small intestine is an important peripheral organ involved in the regulation of food absorption, under the circadian rhythm in normal conditions, it is important to observe its function under different experimental conditions, such as obesity and following physical exercise. Therefore, the aim of this study was to evaluate the effect of regular physical exercise on the morphology of the intestinal mucosa of MSG-obese rats. The morphology, number of goblet cells and IAP expression profile were evaluated. Findings indicate that regular physical exercise causes considerable changes in the parameters evaluated in the intestinal mucosa, reducing some of the effects of MSG-induced obesity on the small intestine.

MATERIAL AND METHODS

Animal Experimental Groups

male Wistar rats with a mean weight of 25 g were obtained from the UEPG animal house and the pups were kept with their mothers until the beginning of the experiments, on the 21th day. The rodents were maintained under conventional conditions with a 12 hr light/dark cycle (lights on at 06:30 hr/lights off at 18:30 hr), with a room temperature between 23°C and 25°C, and received food (nutrition balanced ration from Nuvital, Brazil) and water ad libitum. Twelve animals per group were used for retroperitoneal deposit fat and Lee Index analysis and 3 animals/group for the other parameters. The animals were separated in four groups: one control group, designated as sedentary (S), was not submitted to treatments. The experimental groups submitted to treatments were designated as Monosodium Glutamate (MSG), Exercised (E), and Exercised Monosodium Glutamate (EMSG). All experiments were performed in the morning and after approval by UEPG's Ethic Committee for Animal Experimentation.

Treatments

From day 0 to day 5 of life, each rat in the MSG and EMSG groups received a daily subcutaneous injection of monosodium glutamate (4 g/kg of body weight) according to Lima et al. (2014). The S and E groups received saline injections during the same period of time. From weaning day (21th day) until the 90th day, the E and EMSG groups were submitted to swimming practice for 30 minutes three times a week, beginning at 17:00 h in a swimming bath with water at 32°C. To increase the load during the experimental training period, one weight corresponding to 5% of body weight was tied to the tail of each animal, according to Scomparin et al. (2006).

Determination of the Lee Index

On the 90th day, all rats were anaesthetized with halothane. The body mass and the length from head to caudal region were taken to estimate the Lee Index; calculated as the ratio between the cubic body weight (measured in grams) and skull-caudal length (measured in centimeters) (Bernardis and Patterson, 1968).

Estimate of Retroperitoneal Fat Deposit

On the 90th day before collecting the small intestinal fragments, the retroperitoneal fat was isolated for each rat using manual scraping and immediately weighed; the result was expressed in grams.

Processing of the Intestine

After the measurements had been taken to estimate the Lee Index, three (3) rats were sacrificed by cervical

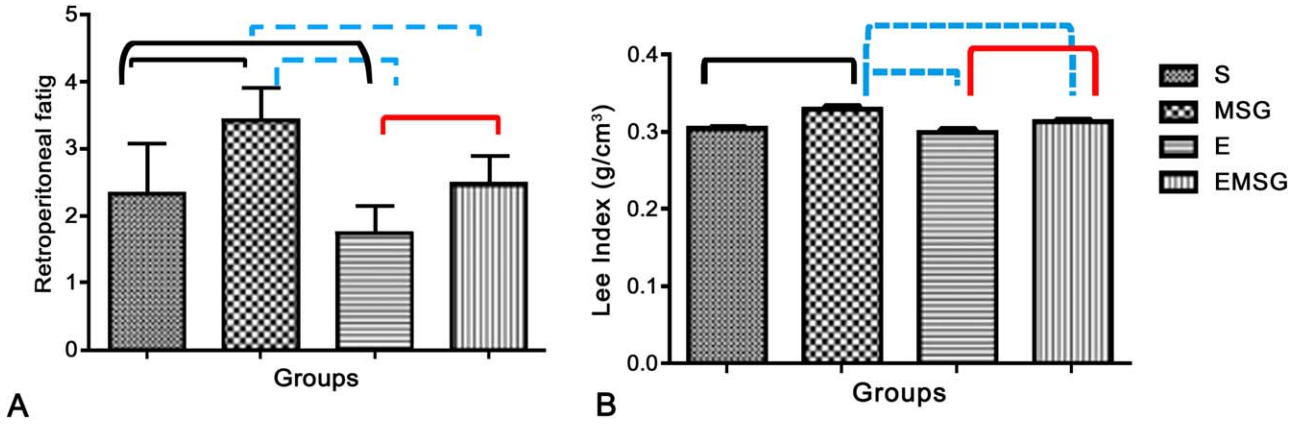

Fig. 1. Mean ± standard deviation of the retroperiotenal fat (g/100g) (A) and in (B) the Index Lee (g/cm3). The full and doted lines indicate the significant differences ($P < 0.05$) among the groups obtained by ANOVA and Tukey post test. S (sedentary), MSG (monossodium glutamate), E (exercised), and EMSG (exercised monossodium glutamate).

dislocation and the jejunum was removed from each rat. The jejunum was sliced into 2-cm long pieces and these were immersed in 2% paraformaldehyde/0.1 M phosphate buffer at pH 7.2 for 48 hr. After fixation, the jejunum samples were dehydrated in an ascending sequence of ethanol and embedded in paraffin. Semi-seriated sections per rat were collected and stained for (immuno)histochemical analysis, as described below. All sections were counterstained with hematoxylin solution.

Goblet Cell Estimate

The slides with sections were immersed in 0.5% periodic acid solution for 15 min and then immersed in Schiff solution for 30 min. For each animal, twenty (20) longitudinally and sectioned villi were selected to count the number of the goblet cells stained on both sides of each villus using a light microscope (Nikon) at a magnification of 50X. The result is expressed as the number of goblet cells per villus. The number of the goblet cells was considered a parameter to infer directly the goblet cell differentiation processes and indirectly, the rate of cell production in the intestinal epithelium in the different treatments.

Intestinal Alkaline Phosphatase (IAP) Detection

The detection of IAP was used as a parameter to evaluate the functionality of the small intestine in the different treatments. Four (4) semi-seriated sections per rat were submitted to immunohistochemical procedures using a Detection System Kit (purchased from SIGMA cod AM0100), according to the manufacturer's instructions. Subsequently, the images for each section were taken at a magnification of 50X, using a microscope and software from Leica and the pixel intensity was determined in 24 areas obtained from the sections per animal and treatment, using the color deconvolution plugin from the Image J software 1.42 (public domain). This plugin converts the immunostaining intensity to pixel values from zero (0) to 255. Thus, for immunohistochemical interpretation, values close to 0 represent higher staining intensities and values close to 255 represent lower staining. Results are expressed as pixel density.

Estimate of Morphometric Parameters

The morphometric parameters were evaluated in images of 10 villi and 10 crypts that were cut longitudinally and obtained from five semi-seriated sections per animal/group, using a Leica microscope. The measurements were performed using the Image J software, which was calibrated using a rule of 100 μm in which the measurements taken in pixels were converted to micrometers for use in all parameters evaluated. The results were expressed in micrometers to estimate the effect of treatments on the intestinal mucosa. The parameters estimated were: (a) thickness of intestinal bowel obtained by the distance from the base of the crypt to the external margin of the longitudinal muscular layer; (b) height of villi, obtained by the distance between the crypt villus junction and the villus tip; (c) thickness of villi, obtained by the distance between the external margins of the villus epithelium measured at the crypt villus junction, and (d) depth of the crypt obtained by the distance between the base of crypt and the crypt villus junction.

Statistical Analyses

All parameters studied were analyzed with the Graphpad Prism software 3.0 using ANOVA with the Tukey post test at $P < 0.05$.

RESULTS

Physical Exercise Significantly Reduces the Retroperitoneal Fat Deposit and Lee Index (LI)

The retroperitoneal fat deposit and Lee index results for the S, MSG, E and EMSG groups are shown in Fig. 1A,B, respectively. The E group presented a significant reduction ($P < 0.05$) in the retroperitoneal fat deposit, when compared to the other groups, including the EMSG group. The retroperitoneal fat deposit in the EMSG group was significantly decreased ($P < 0.05$)

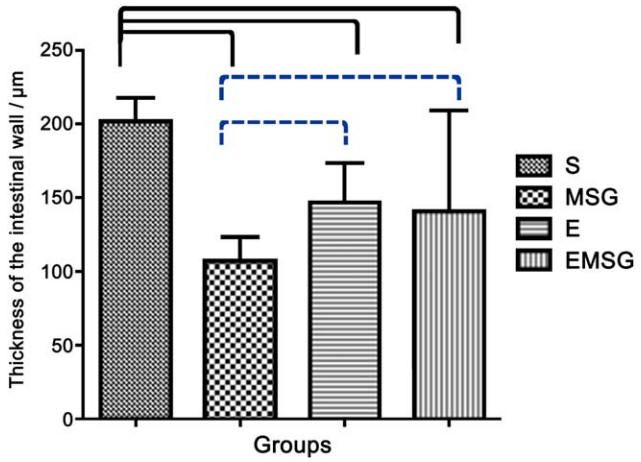

Fig. 2. Mean ± standard deviation of the thickness of the intestinal wall (µm) measured in the jejunum region. The full and doted lines indicate the significant differences ($P < 0.05$) obtained by ANOVA and Tukey post test among the groups S (sedentary), MSG (monossodium glutamate), E (exercised), and EMSG (exercised monossodium glutamate).

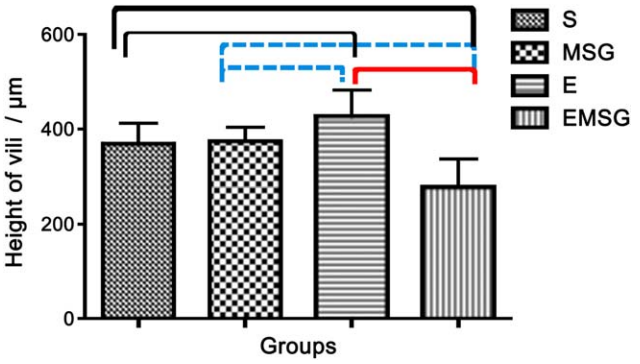

Fig. 3. Mean ± standard deviation of the height of villi measured for the jejunum region and expressed in micrometer (µm). The full and doted lines indicate the significant differences ($P < 0.05$) obtained by ANOVA and Tukey post test among the groups S (sedentary), MSG (monossodium glutamate), E (exercised), and EMSG (exercised monossodium glutamate).

compared to that in the MSG group. The LI was significantly increased in the MSG group ($P < 0.05$), when compared to the other groups. However, in the E group, the LI was significantly reduced ($P < 0.05$), when compared to the MSG and E-MSG groups.

Physical Exercise Significantly Increases the Intestinal Wall Thickness

Figure 2 shows the thickness of the intestinal wall for the different experimental groups. In general, the intestinal wall thickness was significantly reduced in the MSG, E and EMSG groups ($P < 0.05$), in comparison to the S group. However, comparison of the treatment groups showed that the wall thickness in the E group was higher than that in the EMSG and MSG groups. The MSG group presented the thinnest small intestinal wall thickness among the experimental groups.

Physical Exercise Alters Villus Height

Figure 3 depicts the height of villi in the small intestine of rats. In the E group, the villus height was significantly higher ($P < 0.05$) than that of the other groups. No significant difference in villus height was found between the S and MSG groups, whereas the villus height was significantly increased in the E group ($P < 0.05$), when compared to the S, MSG, and EMSG groups. Comparison of all groups revealed a significant reduction in villus height in the EMSG group.

Physical Exercise Increases Villus Thickness and Crypt Depth

Figure 4A shows that villi were slightly thicker in the E group although this difference was not significant when compared to the MSG and S groups; however, the villus thickness was notably increased ($P < 0.05$) in the EMSG group, when compared to the E, MSG and S groups. As far as crypt depth is concerned, the E group demonstrated a significantly higher crypt depth ($P < 0.05$), when compared to the EMSG, MSG, and S groups. The crypt depth in the EMSG group, in turn, was even higher than that in the MSG group and was significantly deeper ($P < 0.05$) than that in the S group.

Physical Exercise Reduces the Number of Goblet Cells

Figures 5A,B show estimates of the number of goblet cells in all groups studied. The number of goblet cells was significantly reduced ($P < 0.05$) in the E group, compared to the S, MSG, and EMSG groups. The number of goblet cells in the MSG group was significantly increased ($P < 0.05$), when compared to all groups. Nonetheless, the number of goblet cells in the EMSG group was significantly increased ($P < 0.05$) compared to the E group.

Physical Exercise Decreases Intestinal Alkaline Phosphatase

Figure 6A shows the intestinal alkaline phosphatase (IAP) activity, expressed as pixels, in the different experimental groups. Figure 6B visually confirms these pixel analyses. In the MSG group, the IAP pixel intensity was significantly decreased ($P < 0.05$), when compared to the S group. However, in the EMSG group, the IAP pixel intensity was significantly reduced ($P < 0.05$), when compared to the other groups.

DISCUSSION

The effect of hypothalamic obesity, induced in rats by continued injections of monosodium glutamate during the first days after birth, causes an increase in the Lee Index and body adiposity in adulthood life, as already established in the literature (Bernardis and Patterson, 1968). Our retroperitoneal fat deposit and Lee index analysis confirmed previous reports regarding the MSG-obese rat model (Bernardis and Patterson, 1968; Mŏzes et al., 2000, 2004) . The present study also demonstrated that the introduction of regular physical exercise in MSG-obese rats, immediately after weaning, was able to

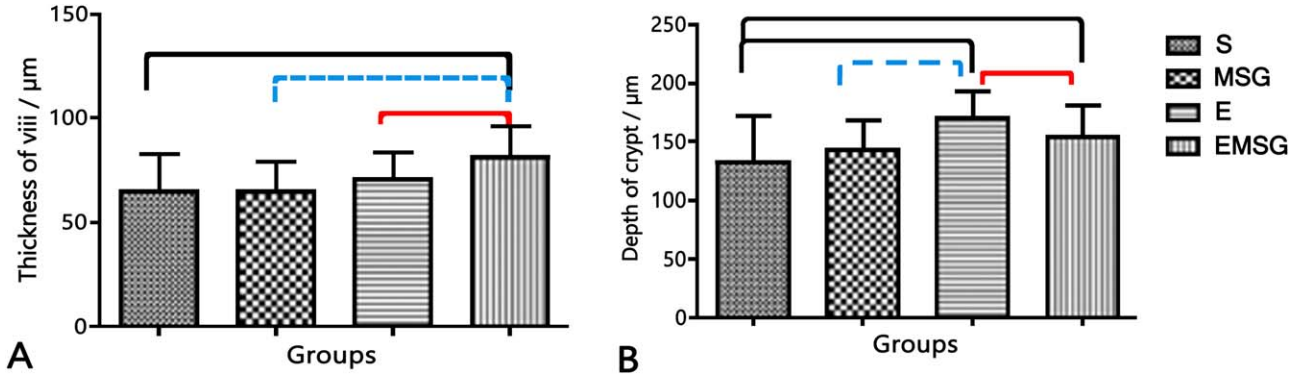

Fig. 4. Mean ± standard deviation of the villi thickness (A) and depth of crypt (B) measured for the jejunum region and expressed in micrometer (µm). The full and doted lines indicate the significant differences ($P < 0.05$) obtained by ANOVA and Tukey post test among the groups S (sedentary), MSG (monossodium glutamate), E (exercised), and EMSG (exercised monossodium glutamate).

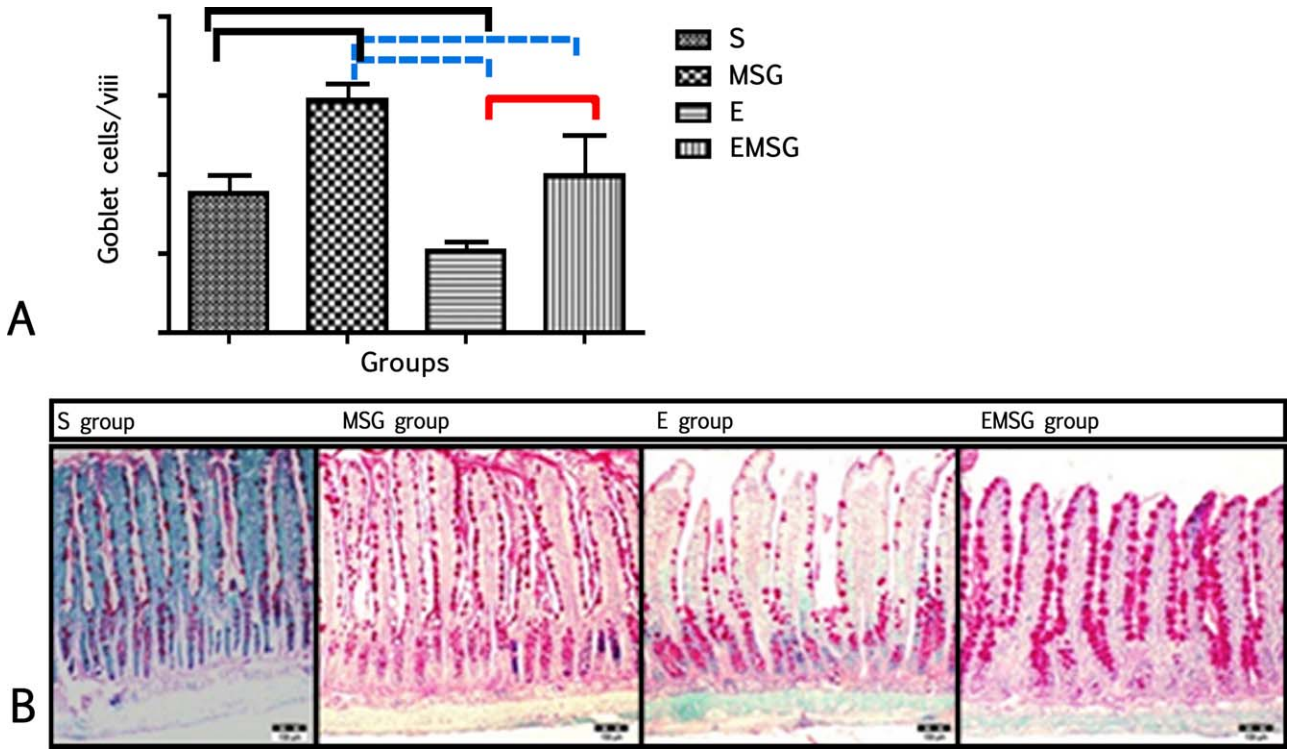

Fig. 5. Mean ± standard deviation for the number of the goblet cells/villi (A) where the full and doted lines indicate the significant differences ($P < 0.05$) obtained by ANOVA and Tukey post test among the groups S (sedentary), MSG (monossodium glutamate), E (exercised), and EMSG (exercised monossodium glutamate). B shows representative photomicrograph of the goblet cells in the jejunum region identified by magenta staining on the villi and into the crypts. Observe that in the MSG and EMSG groups show an increase of the goblet cell while it is reduced in E group.

reduce the retroperitoneal fat deposit and Lee index in adulthood, in accordance with results obtained by Ribeiro et al. (2004). These results indicate that, even after neurons of the arcuate nucleus have been damaged during the first days after birth, physical exercise may stimulate adjustments in the central nervous system in order to minimize the effects of this neuronal injury. However, the exact mechanisms for this adjustment are not well understood yet.

The small intestine is directly involved in the absorption of nutrients and, as it is a peripheral organ, it is also involved in the circadian rhythm. The influence of food on the transition between the suckling diet to a solid diet has been demonstrated during the weaning to

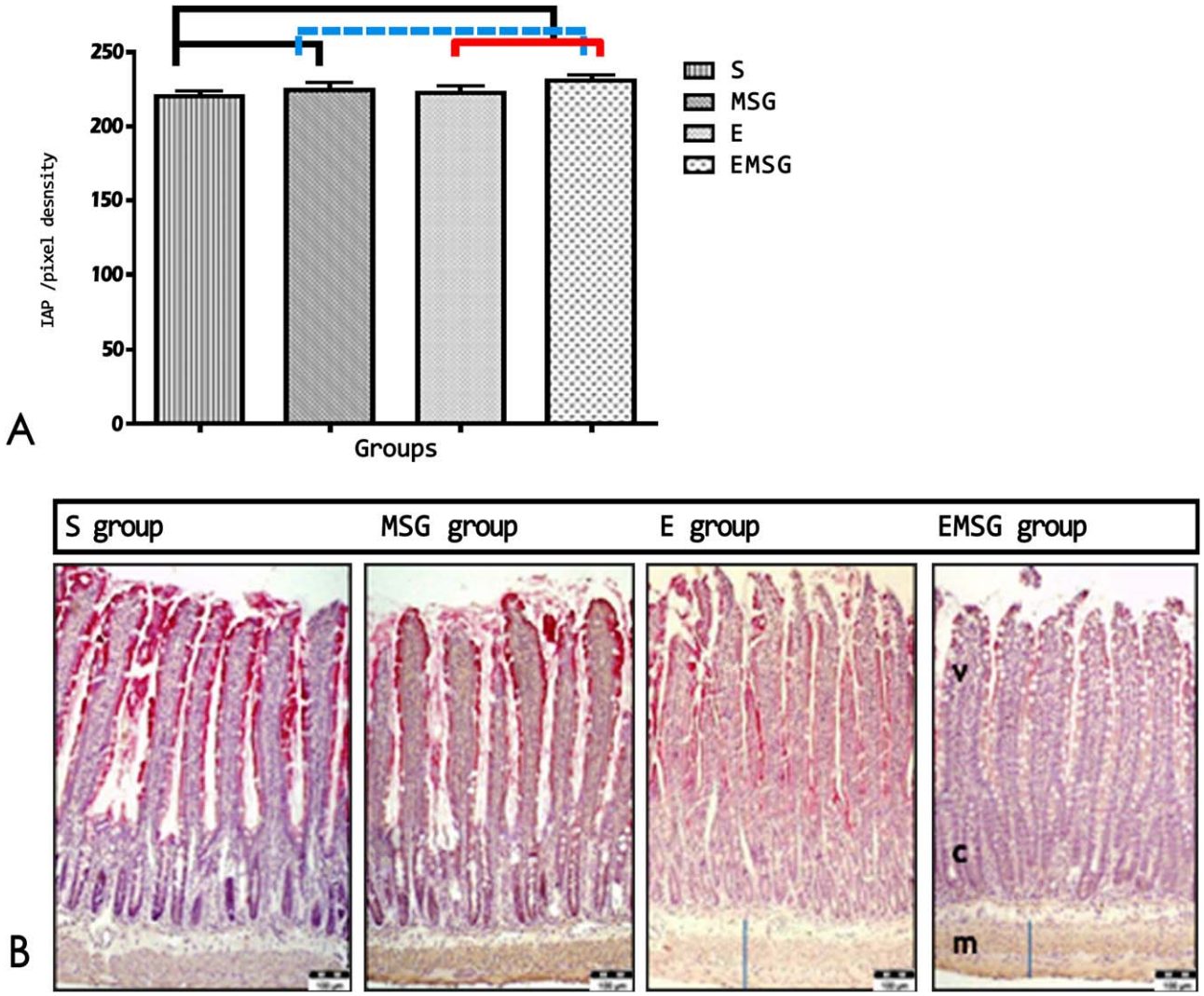

Fig. 6. Mean ± standard deviation of the IAP, expressed in pixel density (A). In B, representative photomicrograph for IAP of the villus of the jejunum region. V = villi; C = crypt; M = muscular layer. In (A) the full and dotted lines indicate the significant differences ($P < 0.05$) obtained by ANOVA and Tukey post test among the groups S (sedentary), MSG (monossodium glutamate), E (exercised), and EMSG (exercised monossodium glutamate).

adulthood phase (Soares et al., 2009). Thus, during this transition phase, several hormones produced by different parts of the digestive system can affect the hypothalamus, the control of satiety and the growth hormone production (Suzuki et al., 2010). Although the exact mechanism is not understood, previous results from Hamaoka and Kusunoki (1986) revealed an increase in cell proliferation in the small intestine of MSG-obese rats. In the present study, determining the goblet cell numbers allowed us to infer about the cell proliferation in the small intestine. The number of goblet cells was increased in the MSG group, but no concomitant increase in villus height or crypt depth was observed. In terms of cell kinetics, it is possible that in conditions of obesity, the cell proliferation occurring in the crypts leads to an increase in the length of the small intestine, as proposed by Hamaoka and Kusunoki (1986). Therefore, the present results suggest that in MSG obesity, a higher rate of cell production in the crypts could produce an increase in differentiated goblet cells, as observed in the villi. An increase in the number of goblet cells - and consequently in mucus - during obesity may be important for protective functions, according to previous reports. Maynard et al. (2012) reported that mucus in the small intestine may protect against microbiota products. These authors suggested that the presence of MAMP lipopolysaccharide or peptidoglycan stimulates mucus production, with different compositions, in germ-free mice. Butyrate, produced by benign constituents of the microbiota, also promotes increased mucin release, providing a positive-feedback loop for maintenance of the mucus barrier and its colonization by butyrate-producing commensals. The importance of the mucus layer is evident in mucin-deficient mice (deficient in Muc2); these mice have increased translocation of commensal and pathogenic bacteria and spontaneously

develop colitis (Maynard et al., 2012). Thus, the increase in the number of goblet cells observed in the MSG group may be related to their protective effect against some microbiota products during obesity.

Importantly, the present study showed for the first time that physical exercise decreases the number of goblet cells in the small intestine; this finding is perhaps not unexpected since a larger number of absorptive cells (enterocytes), compared to goblet cells, are required during physical activity. However, when physical exercise was associated with obesity, the number of goblet cells remained decreased, indicating that physical exercise had a positive effect on the cell kinetics of the intestinal epithelium. Taken together, data suggest that physical activity stimulates cell proliferation into the crypts and the differentiation of absorptive cells instead of goblet cells, possibly contributing to the increase in crypt depth and villus thickness observed in our results. We postulate that the increase in crypt depth and villus thickness may occur when the steady state of cell kinetics in the intestine is lost. This would be possible if the loss of a few cells from the villi is compensated by a concomitant increase in cell proliferation into the crypts. As such, cell migration to the crypt villus junction may have led to the increased villus thickness observed, as the small intestine is an organ that undergoes physiological adaptation according to the diet during its growth, altering cell proliferation, migration and circadian rhythm (Gomes and Alvares, 1998; Wille et al., 2004; Gomes et al., 2005; Soares et al., 2009). In addition, we suggest that the reduction in goblet cell numbers, caused by physical exercise, may also be related to a change in microbiota in the small intestine that could differ between states of obesity and exercise; however, the hypothesis that physical exercise may change the microbiota of the small intestine needs to be further explored.

Intestinal alkaline phosphatase activity was found to be reduced in both the obesity and physical exercise groups since our data were obtained during the light/daytime period in all groups. The IAP activity in the small intestine observed in the MSG group during the light period, was in accordance with data obtained by Martinková et al. (2000), who demonstrated that there is a circadian rhythm for IAP activity in the small intestine of MSG-obese rats, with higher activity during the dark period of the day and decreased activity during the light period.

This study is the first to demonstrate reduced IAP activity by physical exercise during obesity. This observation may be explained by the function of IAP, as recently described by Koyama et al. (2002), Bates et al. (2007), Geddes and Philpott (2008), and Maynard et al., (2012), since it is thought to be related to the microbiota present in the intestinal lumen. According to these authors, IAP breaks down the lipopolysaccharides produced by different microbiota present in the lumen of the small intestine, protecting the body against their absorption. The lipopolysaccharides, after passing across the intestinal epithelium, induce a local inflammation, and the subsequent cytokine production augments insulin resistance, leading the body towards a condition of obesity (Ley et al., 2005; Armougom and Raoult, 2008; Ducan et al., 2008; Herbert and Arthur, 2011). We hypothesize that a possible change in the intestinal microbiota, induced by physical exercise, could also reduce IAP activity, indicating a protective effect of physical exercise against microbiota products during obesity.

Finally, our results demonstrate that physical activity, alone or in association with obesity, reduces fat deposits, body mass index, goblet cell numbers and IAP activity. We conclude that regular physical exercise may modulate, via an unknown mechanism, the functional relationship between the small intestine and the hypothalamic center in the MSG obesity model.

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