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Effects on secretory IgA levels in small intestine of mice that underwent moderate exercise training followed by a bout of strenuous swimming exercise

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ABSTRACT

Intestinal homeostasis effectors, secretory IgA (SIgA) and polymeric immunoglobulin receptor (pIgR), have been evaluated in proximal and distal small intestine with moderate-exercise training but not with strenuous exercise or a combination of these two protocols. Therefore, two groups of mice (n = 6–8) were submitted to strenuous exercise, one with and one without previous training. The control group had no exercise protocol. Assessment was made of intestinal SIgA and plasma adrenal hormones (by immunoenzymatic assay), alpha-chain and pIgR proteins in intestinal mucosa (by Western blot), lamina propria IgA plasma-cells (by cytofluorometry), mRNA expression (by real-time PCR) for pIgR, alpha- and J-chains in liver and intestinal mucosa, and pro- and anti-inflammatory cytokines in mucosa samples. Compared to other exercise protocols, training plus strenuous exercise elicited: (1) higher levels of SIgA and pIgR in the proximal intestine (probably by hepatobiliary contribution); (2) higher levels of SIgA in the distal segment; (3) lower mRNA expression of some SIgA- and most pro-inflammatory pIgR-producing cytokines. SIgA and pIgR in both segments were derived from an existing pool of their corresponding producing cells. The apparent decreased translation of mRNA transcripts underlies lower levels of SIgA and pIgR in distal than proximal small intestine. There was no significant difference in the relatively high adrenal hormone levels found in both exercised groups. Further study is required about the effects of training plus strenuous exercise on pool-derived SIgA levels and mRNA expression of pro-inflammatory pIgR-producing cytokines. These results could have important implications for intestinal disorders involving inflammation and infection.

1. Introduction

Acute exercise is defined as a single bout of strenuous activity, whereas exercise training consists of multiple exercise sessions (Kispert and Nielsen, 1985). By means of stress adrenal hormones (i.e., epinephrine), these two exercise protocols cause short-term and long-term changes, respectively, on mucosal immunity, modifying the production of secretory IgA (SIgA), the levels of cytokines, and the activation, viability and distribution of lymphocytes (Walsh et al., 2011). It is known that when training involves moderate exercise, the associated stress can induce beneficial effects on humans and animals. Contrarily, the stress caused by strenuous exercise can lead to immunosuppressive and pro-inflammatory effects.

Experimental studies have evidenced that a single bout of acute exercise causes immunosuppressive effects that are partially mediated by the increasing levels of adrenal hormones, such as corticosterone and adrenaline. Such effects are evidenced by modulation of mucosal T lymphocyte traffic (Kruger et al., 2008; Quadrilatero et al., 2003), reduction of salivary IgA production (Kimura et al., 2008), suppression of the production of antigen-specific Th1 (interferon gamma) and Th2 (interleukin 10) cytokines in response to upper respiratory infection (Kohut et al., 2001), and less cell viability due to increased apoptosis, both in intestinal lymphocytes (Hoffman-Goetz and Quadrilatero, 2003; Marra et al., 2005) and submandibular lymphocytes (Quadrilatero et al., 2003).

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The prolonged practice of moderate exercise, on the other hand, has been shown in mouse models to provide beneficial effects, evidenced by increased cell viability resulting from prevention of apoptosis (Avula et al., 2001), greater antigen specific antibody responses (Kaufman et al., 1994), and protection against oxidative colonic injury (Hoffman-Goetz et al., 2009; Kasimay et al., 2006). Additionally, moderate exercise training can attenuate the deleterious effects of acute stress prompted by a single bout of strenuous exercise, which include a loss of lymphocytes and an exaggerated inflammatory response and/or oxidative stress response (Boudreau et al., 2005; Davidson and Hoffman-Goetz, 2006; Fu et al., 2003; Hoffman-Goetz et al., 2010).

Likewise in humans, the practice of moderate exercise has shown beneficial effects, evidenced in one study by the up-regulation of salivary IgA levels (Klentrou et al., 2002), whereas acute stress caused by intensive exercise has proven immunosuppressive, evidenced by decreased levels of salivary SIgA (Laing et al., 2005; Mackinnon et al., 1993; Tharp and Barnes, 1990) which can represents a contributing risk factor for upper respiratory diseases (Neville et al., 2008; Tiollier et al., 2005). Indeed, salivary SIgA is regarded as a health biomarker to evaluate the risk of upper respiratory infections associated with acute and chronic exercise in athletes (Bishop and Gleeson, 2009).

In the intestinal mucosa, production of SIgA and the pIgR-mediated transport of SIgA (Harriman et al., 1999; Johansen et al., 1999; Shimada et al., 1999; Uren et al., 2003) have an essential role in protection against bacterial invasion and maintenance of homeostasis. The production of SIgA, representing the downstream event regulated by cytokines, results from class switching recombination (CSR) of either T-cell dependent or independent activation of B cells. T-cell activation of B cells takes place in Peyer's patches, while T-cell independent B cell generation occurs in isolated follicles and lamina propria of the intestinal mucosa, leading in each case to the subsequent maturation of these cells (Cerutti and Rescigno, 2008; Suzuki and Fagarasan, 2009). During their differentiation, B cells change the expression pattern of their chemokine and integrin receptors, which lead them to the intestinal lamina propria, where they are differentiated into IgA+ plasma cells (Kunkel and Butcher, 2003). These IgA+ plasma-blasts release IgA antibodies, mainly in the form of a dimer (dIgA) or of an oligomer joined by the J-chain. The latter is a small polypeptide of 15 KDa also produced by these IgA secreting cells (Johansen et al., 2001). The J-chain interacts with the polymeric immunoglobulin receptor (pIgR) to assemble monomeric IgA. The pIgR is an antibody transport protein expressed on the basolateral surface of columnar cells of the intestinal mucosal epithelium (Brandtzaeg, 1974). IgA-J chain complexes are captured and internalized by pIgR after being released in the lamina propia. SIgA trancytosis via pIgR then transports them to the apical plasma membrane. Once plgR arrives to the apical surface, a portion of it is cleaved and released in the form of the secretory component (SC). This component and dimeric IgA form SIgA.

Taking into account that SIgA production is a regionalized event in the small intestine, some studies have addressed the effects of stress induced by moderate exercise on the production of SIgA in duodenum (Viloria et al., 2011) and ileum (Drago-Serrano et al., 2012). However, there is scant evidence of the effects of acute stress, provoked by an intensive exercise session or, of moderate exercise training followed by a session of intensive exercise, on the pIgR-mediated transport and production of SIgA in the small intestine. This information could provide important insights into the effects of these exercise protocols, as well as to the mechanisms of adaptation to stress, since it is known that this transport function is vital for intestinal homeostasis and protection of the organism against bacteria (Corthesy, 2007; Phalipon and Corthesy, 2003). Thus, the aim of the present study was to evaluate, in the proximal and distal regions of mouse small intestine, the SIgA and pIgR production that results from strenuous exercise in groups of animals with and without previous moderate training.

2. Materials and methods

2.1. Animals

Two-month old male Balb/c mice were obtained from Harlan Sprague Dawley, Inc. They were housed 1 per cage in a room with little noise and were fed with the NIH-31 diet *ad libitum*. The animals were kept on a 12:12 h light/dark cycle (lights on at 6 am). All handling and assays were carried out between 8 and 11 am to avoid the influence of the circadian cycles of the adrenocorticotropic hormone (ACTH) and corticosterone. Animals were handled in accordance with Mexican federal regulations for animal experimentation and care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico City, Mexico) and approved by the Institutional Animal Care and Use Committee. Mice were conditioned to their environment before being submitted to the exercise protocol described below.

2.2. Experimental groups and exercise protocol

Mice were randomly divided into four groups (6–8 animals per group). One group was sedentary (no exercise). The second group was submitted to a two-week period of adaptation to swimming exercise only during the 27th and 28th weeks of life. The third group was submitted to the same adaptation protocol, also during the 27th and 28th weeks of life, but in this case followed by a single bout of strenuous swimming exercise in the 29th week. This group represents strenuous exercise without any previous training. The adaptation protocol is the minimum preparation required for mice to undergo swimming exercise of 5.5 h without causing necrosis and hemorrhage in the small intestine, or in the worst of cases death (which by previous experience was found to take place when sedentary mice were submitted to this same strenuous exercise protocol). The fourth group was submitted to a two-week period of adaptation to swimming exercise during the 9th and 10th week of life, followed by 18 weeks of moderate swimming exercise training (in which the mice swam 60 min every other day, three times per week) up to the 28th week of life, and finally a single bout of strenuous swimming exercise in the 29th week. This group represents strenuous exercise with previous moderate exercise training. All animals were sacrificed in the 29th week of life to avoid variations in effects based on age.

During the two-week adaptation period, the time of swimming sessions was gradually increased. On the first day the mice swam for 10 min, followed by daily increments of 5 min until the swimming session reached 60 min. The final bout of strenuous swimming exercise represented acute stress. 5.5 h was chosen for acute stress, because in a preliminary assay mice refused to swim after this period in spite of being softly stimulated by a finger.

For training sessions, each mouse was placed in a clear plastic container half full of water. While swimming, the mice could see outside, see other mice swimming, and orient themselves to their environment. Water temperature was 30–32 °C. After each swimming session, mice were dried with a towel to avoid suddenly changes in body temperature. In case of floating behavior, the mouse was softly pushed with a finger, which was sufficient stimulus with a minimum of additional stress (Kregel, 2006).

2.3. Collection of biological samples

After the session of strenuous exercise that was employed for two-groups, all four groups were immediately anesthetized with diethyl-ether and exsanguinated by cardiac puncture. Plasma