Experimental Physiology

Colitis-induced oxidative damage of the colon and skeletal muscle is ameliorated by regular exercise in rats: the anxiolytic role of exercise

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Epidemiological studies have shown that exercise protects the gastrointestinal tract, reducing the risk of diverticulosis, gastrointestinal haemorrhage and inflammatory bowel disease, while many digestive complaints occurring during exercise are attributed to the adverse effects of exercise on the colon. In order to assess the effects of regular exercise on the pathogenesis of colitis, Sprague–Dawley rats of both sexes were either kept sedentary or given exercise on a running wheel (0.4 km h^{-1}, 30 min for 3 days $week^{-1}$). At the end of 6 weeks, under anaesthesia, either saline or acetic acid (4%, 1 ml) was given intracolonically. Holeboard tests were performed for the evaluation of anxiety at 24 h before and 48 h after induction of colitis. Increased 'freezing time' in the colitis-induced sedentary group, representing increased anxiety, was reduced in the exercised colitis group ($P < 0.05$). On the third day following the colonic instillation, the rats were decapitated under brief ether anesthesia and the distal 8 cm of the colons were removed. In the sedentary colitis group, macroscopic and microscopic damage scores, malondialdehyde level and myeloperoxidase activity were increased when compared to the control group ($P < 0.01$–0.001), while exercise prior to colitis reduced all the measurements with respect to sedentary colitis group ($P < 0.05$–0.001). The results demonstrate that low-intensity, repetitive exercise protects against oxidative colonic injury, and that this appears to involve the anxiolytic effect of exercise, suggesting that exercise may have a therapeutic value in reducing stress-related exacerbation of colitis.

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A substantial body of evidence verifies the benefits of regular physical activity on physiological and psychological wellbeing (Blair *et al.* 1989; Fletcher *et al.* 1992). Physical activity is beneficial to health because it reduces the risk of cardiovascular and endocrine diseases, improves bone and muscle conditioning, and lessens anxiety and depression (Bi & Triadafilopoulos, 2003). However, depending partly on the training intensity (Simren, 2002), the impact of exercise on the gastrointestinal system has been either beneficial or harmful (Bi & Triadafilopoulos, 2003). In particular, regular exercise at low intensity may reduce the risk of gastrointestinal disorders such as colonic cancer, cholelithiasis, diverticular disease or constipation.

Ulcerative colitis (UC) is an idiopathic inflammatory bowel disease (IBD) with diffuse, recurrent inflammation of the colon and rectum, which is predominantly characterized by cycles of acute inflammation, ulceration and bleeding of the colonic mucosa (Sanahan, 1993). The aetiology of UC is not clearly understood, but inflammatory mediators, such as cytokines and eicosanoids, and excessive production of reactive oxygen species (ROS) by the inflamed mucosa have been proposed to contribute significantly to the development of tissue injury (Babbs, 1992). In contrast, there is a long history of observations suggesting that psychological stress contributes to the course of IBD (Maunder, 2005) and that chronic stress increases the severity of intestinal inflammation (Gulpinar *et al.* 2004). Considering the improvements in psychological outcomes (i.e. reduction of depression and anxiety, and improved mood states) reached by habitual exercise (Byrne & Byrne, 1993),

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a number of studies have investigated the preventive effect of physical activity on inflammatory bowel diseases. Sonnenberg (1990) has shown that sedentary and physically less demanding occupations were associated with a higher risk of inflammatory bowel disease than physically demanding occupations. In contrast, the results of a case control study comparing incidence rates of IBD showed a reduced risk for physically active patients with Crohn's disease (CD) or UC, while in another study active CD patients had a high risk (Klein *et al.* 1998; Sorensen *et al.* 1987). Suggested underlying mechanisms in the preventive effect were the stress reducing effects of physical activity and changes in local neuro-immuno-endocrine effects (Loudon *et al.* 1999).

Although there is a large amount of research examining the impact of exercise on the gastrointestinal tract, most of the clinical studies conducted in humans had methodological limitations, making it difficult to explore the mechanisms involved in the potential benefits or hazards of physical activity on the intestinal tissue. Regarding the presence of insufficient information on the effects of regular exercise on the pathogenesis of colitis, the primary purpose of the present study was to examine the potential anxiolytic and protective effects of exercise in the underlying mechanisms of oxidative colonic injury. Another purpose of the study was to examine the effect of colonic injury on skeletal muscle, which was preconditioned to oxidative injury during the exercise training.

Methods

Animals

Adult Sprague–Dawley rats of both sexes ($n = 18$) weighing 250–300 g were housed individually in a light- and temperature-controlled room on a 12 h–12 h light–dark cycle, in which the temperature ($22 \pm 2^{\circ}$C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet lab chow, and food was withdrawn overnight before induction of colitis, but access to water was allowed *ad libitum*. The animals were randomly divided into three groups as: control (non-trained non-colitis-induced; $n = 6$), non-runner colitis ($n = 6$) and runner colitis groups ($n = 6$). The experiments were approved by the Marmara University School of Medicine Animal Care and Use Committee.

Training protocol

A rotarod (Northel, İstanbul, Turkiye) with a 7 cm diameter rod and a constant acceleration capability (1 cm s^{-2}) was used. The rod was elevated 40 cm above the floor of the cage. Before daily training, rats were allowed to accommodate on the rod for 5 min. Runner rats were subjected to low-intensity (7 m min^{-1}; 30 min day^{-1}; 3 days $week^{-1}$) training for 6 weeks (Mitchell *et al.* 2004). During the running session, if the rat fell off the rod, a sensor was activated to stop rotation. Then the rat was put back on the rod until 30 min running time was completed. After the first week of training, rats were accustomed to run without any falls. Non-runner rats remained in their cages in the experiment room during the running period and were handled for an equal amount of time.

Induction of experimental colitis

At the end of 6 weeks and 1 h after the last exercise session, induction of colitis was performed. Before the induction of colitis, the rats were deprived of food, but not water, for 18 h. Colitis was induced by a modification of the method of MacPherson & Pfeiffer (1978). The induction of colitis was performed by intracolonic administration of 1 ml of 4% (v/v) acetic acid diluted in saline (pH 2.3) through a polyethylene tube (PE-60), the tip of which was positioned in the colon 8 cm past the anus under light ether anaesthesia. Acetic acid was slowly administered into the colonic lumen using ether inhalation. After a 30 s period of exposure, excess fluid was withdrawn, and the colon was then flushed with 1.5 ml of phosphate-buffered saline (pH 7.4). Control animals were subjected to the same procedure with the exception that isotonic saline was substituted for acetic acid.

Evaluation of anxiety

It is well known that an increase in anxiety reduces exploratory behaviour in rats, which can be tested by using the holeboard test (Marco *et al.* 2005). The holeboard apparatus, providing a measure of directed exploration in rats (File & Wardill, 1975), consisted of a wooden board (40×40 cm) with 16 equally spaced holes (each 13 cm in diameter).

The holeboard test was performed by placing the rat in the centre of the wooden board, and the test was recorded by a video camera for 5 min. Then the 'freezing time', when no movement of the trunk, head or extremities was observed was counted from the videotape (Boissier & Simon, 1962). Increased freezing time (as a percentage over 5 min) indicated a reduction in the exploratory behaviour and increased anxiety.

The holeboard test was first performed in both the runner and non-runner rats on the last day of the 6 week period before the induction of colitis. The test was repeated during the forty-eighth hour following acetic acid instillation.

Assessment of tissue injury

Macroscopic scoring The rats were decapitated 72 h after the colonic instillation of saline or acetic acid, and the distal 8 cm of colons were opened down their

mesenteric borders and cleansed of luminal contents. The severity of gross macroscopic damage in the colon was then graded using the following criteria modified from Wallace *et al.* (1992): 0, normal appearance; 1, focal hyperaemia, no ulcers; 2, single site of ulceration without associated inflammation; 3, single site of ulceration with inflammation 4, two or more sites of discrete ulceration and inflammation; 5, major site of injury or inflammation extending 1–2 cm along length of colon; and 6–10, score increased by one for each additional centimeter of damage or injury beyond 2 cm.

Biochemical measurements. Colon and gluteus muscle obtained from each animal were stored at –80°C until the determination of tissue myeloperoxidase (MPO) activity, lipid peroxidation (LP) and glutathione (GSH) levels. Tissue MPO activity is frequently utilized to estimate tissue neutrophil accumulation in inflamed tissues and has previously been shown to correlate significantly with the number of neutrophils determined histochemically in colonic tissues (Bradley *et al.* 1982). The method of assay of MPO activity in the present study was similiar to that previously described by others (Bradley *et al.* 1982). The tissue samples (0.2–0.3 g) were homogenized in 10 volumes of ice-cold potassium phosphate buffer (50 mM K_2HPO_4, pH 6.0) containing hexadecyltrimethylammonium bromide (HETAB; 0.5%, w/v). The homogenate was centrifuged at 41 400 *g* for 10 min at 4°C, and the supernatant was discarded. The pellet was then rehomogenized with an equivalent volume of 50 mM K_2HPO_4 containing 0.5% (w/v) HETAB and 10 mM EDTA (Sigma). Myeloperoxidase activity was assessed by measuring the H_2O_2-dependent oxidation of *o*-dianizidine.2HCl. One unit of enzyme activity was defined as the amount of MPO present per gram of tissue weight that caused a change in absorbance of 1.0 min^{-1} at 460 nm and 37°C.

Samples of colonic and muscle tissue were homogenized in 10 volumes of ice-cold 10% trichloracetic acid in an Ultra Turrax tissue homogenizer. Homogenized tissue samples were centrifuged at 2000 *g* for 15 min at 4°C. The supernatant was removed and recentrifuged at 41 400 *g* for 8 min. Gluthatione measurements were performed using a modification of the Ellman procedure (Aykaç *et al.* 1985). Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid-reactive substances as previously described (Casini *et al.* 1986). Lipid peroxide levels were expressed in terms of malondialdehyde (MDA) equivalents using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Histological analysis. For light microscopic investigations, samples from distal colon and gluteus muscle were fixed in 10% buffered *p*-formaldehyde and prepared for routine paraffin embedding. Sections of tissue were cut at 5 μm on a rotary microtome, mounted on slides, stained with Haematoxylin and Eosin (H&E), and examined under an Olympus BH 2 photomicroscope. All tissue sections were examined microscopically for characterization of histopathological changes by an experienced histologist (F.E.) who was unaware of the treatment conditions. Assessment of the colonic injury was performed using the previously described criteria: damage/necrosis (0, none; 1, localized; 2, moderate; 3, severe); submucosal oedema (0, none; 1, mild; 2, moderate; 3, severe); inflammatory cell infiltration (0, none; 1, mild; 2, moderate; 3, severe); vasculitis (0, none; 1, mild; 2, moderate; 3, severe); and perforation (0, absent; 1, present), with a maximum score of 13 (Gue *et al.* 1997). The histological analysis of the skeletal muscle was made using the criteria: disorganization of the muscle fibres (0, none; 1, mild; 2, moderate; 3, severe); and inflammatory cell infiltration (0, none; 1, mild; 2, moderate; 3, severe), with a maximum score of 6 (Erkanli *et al.* 2005).

Statistical analysis

The results are expressed as means ± S.E.M. with six rats per group. Instat statistical package (GraphPad Software, San Diego, CA, USA) was used. Following the assurance of normal distribution of data, one-way analysis of variance (ANOVA) was used for multiple comparisons and unpaired Student's *t* test was used to evaluate the level of statistical significance between two groups. Differences were considered statistically significant if $P < 0.05$.

Results

In the non-colitis groups instilled with saline, the recorded freezing time on the holeboard platform was significantly reduced in the runner group when compared with that of the non-runner rats, indicating the anxiolytic effect of exercise training ($P < 0.05$; Fig. 1). In the acetic acid-instilled rats without pretraining, freezing time was increased compared to the non-colitis sedentary group ($P < 0.05$), while colitis-induced exaggeration of anxiety was abolished in trained rats ($P < 0.05$).

Since none of the biochemical or histological parameters was different between the runner and non-runner control rats with intracolonic saline instillation, the non-runner control group was chosen for the presentation of data. Macroscopically scored colonic damage was increased in the non-runner group with colitis when compared to the control group ($P < 0.01$), while exercise prior to colitis reduced the damage score significantly ($P < 0.05$; (Fig. 2*A*). Similarly, the microscopic score in the non-runner colitis group was significantly higher than that of the control group ($P < 0.001$), with the rats that had

exercise prior to colitis induction having scores between these two groups ($P < 0.01$ compared to control and non-runner colitis groups; Fig. 2*B*).

Colonic MPO activity was increased significantly after the induction of colitis when compared to the control group ($P < 0.001$), while exercise prior to colitis reduced the MPO activity significantly compared to the non-runner colitis group ($P < 0.05$; Fig. 3*A*). Interestingly, the induction of colitis elevated the MPO activity in the gluteus muscle of non-runners ($P < 0.001$), while exercise-trained rats with colitis had significantly reduced MPO activity in the muscle compared to the non-runner colitis group ($P < 0.001$; Fig. 3*B*).

Malondialdehyde levels in both the colon and muscle were increased significantly after the induction of colitis when compared to the control group ($P < 0.01$ and $P < 0.001$, respectively), while MDA levels were reduced significantly in the runner rats with colitis compared to the non-runner colitis group ($P < 0.001$; Fig. 4*A* and *B*). In the sedentary group, the GSH level in the muscle was decreased significantly after the induction of colitis ($P < 0.01$), while in rats that had regular exercise prior to colitis muscle GSH levels were replenished ($P < 0.05$; Fig. 5*B*). However, the induction of colitis in non-runners increased the level of colonic GSH content in comparison to the control group ($P < 0.01$), while exercise prior to colitis abolished colitis-induced elevation in the colonic GSH level ($P < 0.001$; Fig. 5*A*).

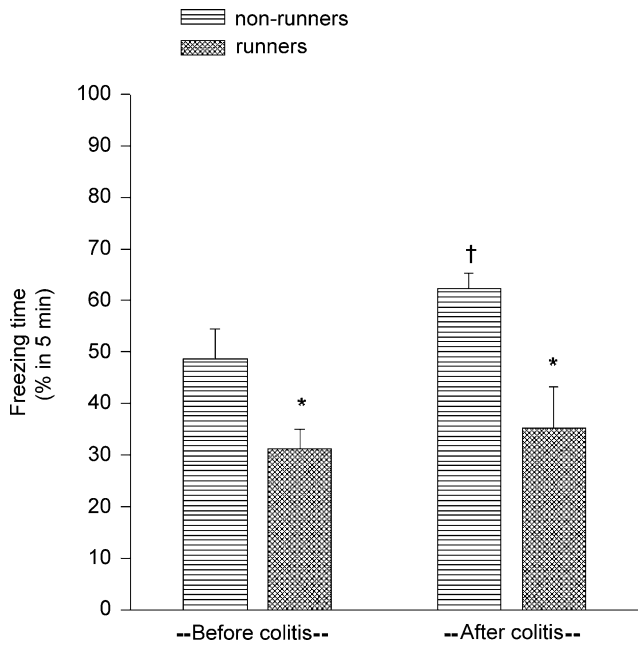

Figure 1. Recorded freezing time as a percentage of total time (5 min) the rats spent on the holeboard platform
In both runner and non-runner rats, holeboard tests were peformed on two occasions, 24 h before and 48 h after induction of colitis. *$P < 0.05$ compared to corresponding non-runner group; †$P < 0.05$ compared to non-colitis non-runner group.

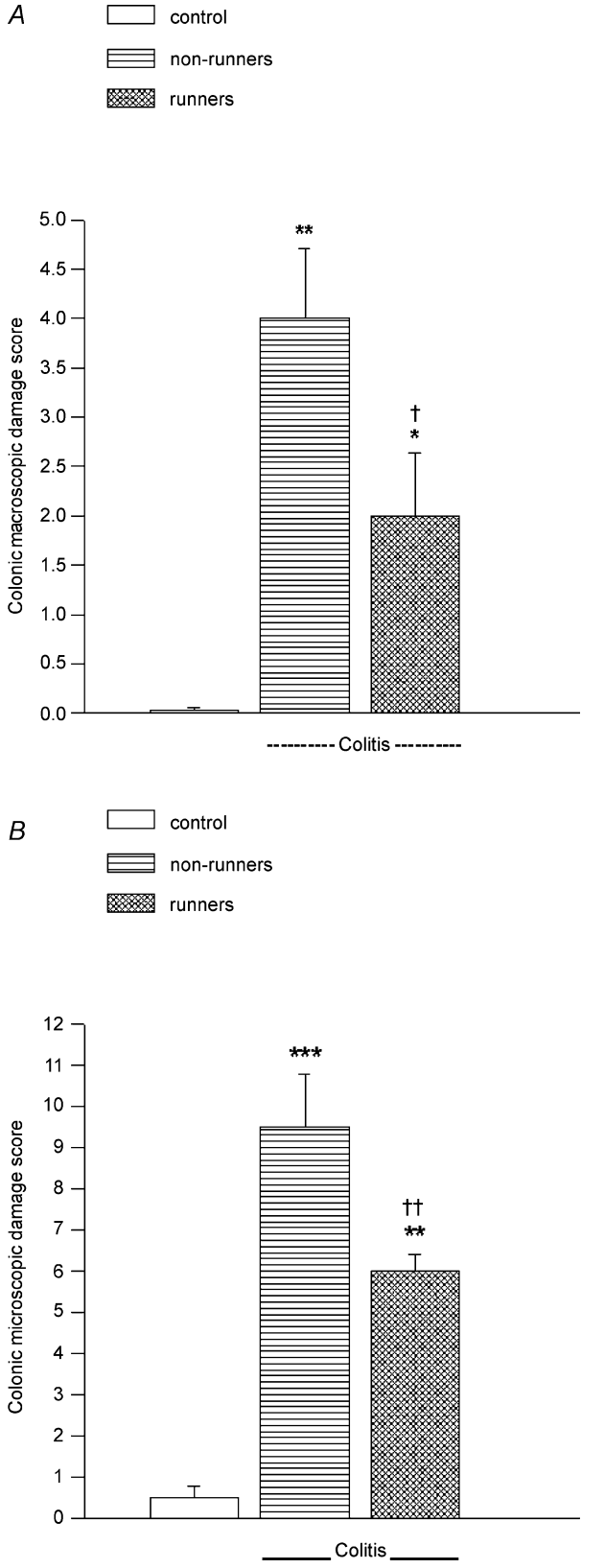

Figure 2. Colonic macroscopic (*A*) and microscopic damage scores (*B*)
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to control group; †$P < 0.05$, ††$P < 0.01$ compared to corresponding non-runner group.

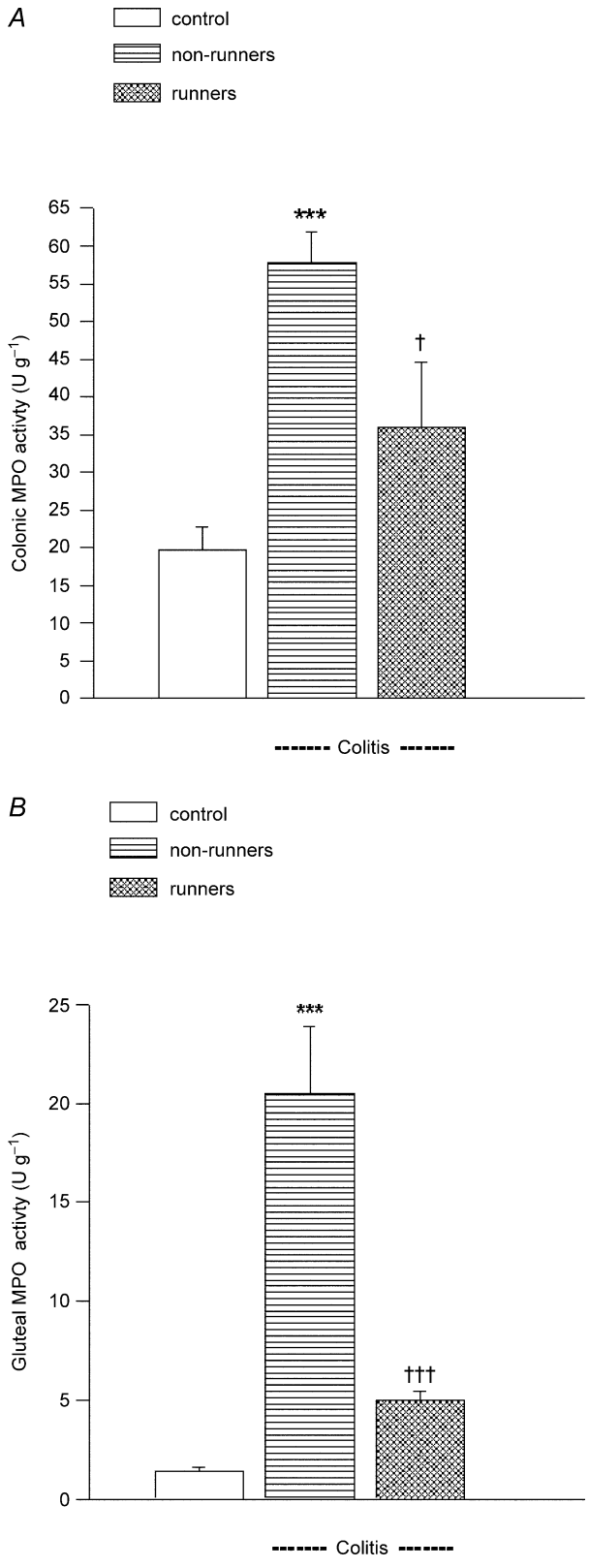

Figure 3. Myeloperoxidase (MPO) activity of colonic (*A*) and gluteus muscle tissues (*B*)
***$P < 0.001$ compared to control group; †$P < 0.05$, †††$P < 0.001$ compared to corresponding non-runner group.

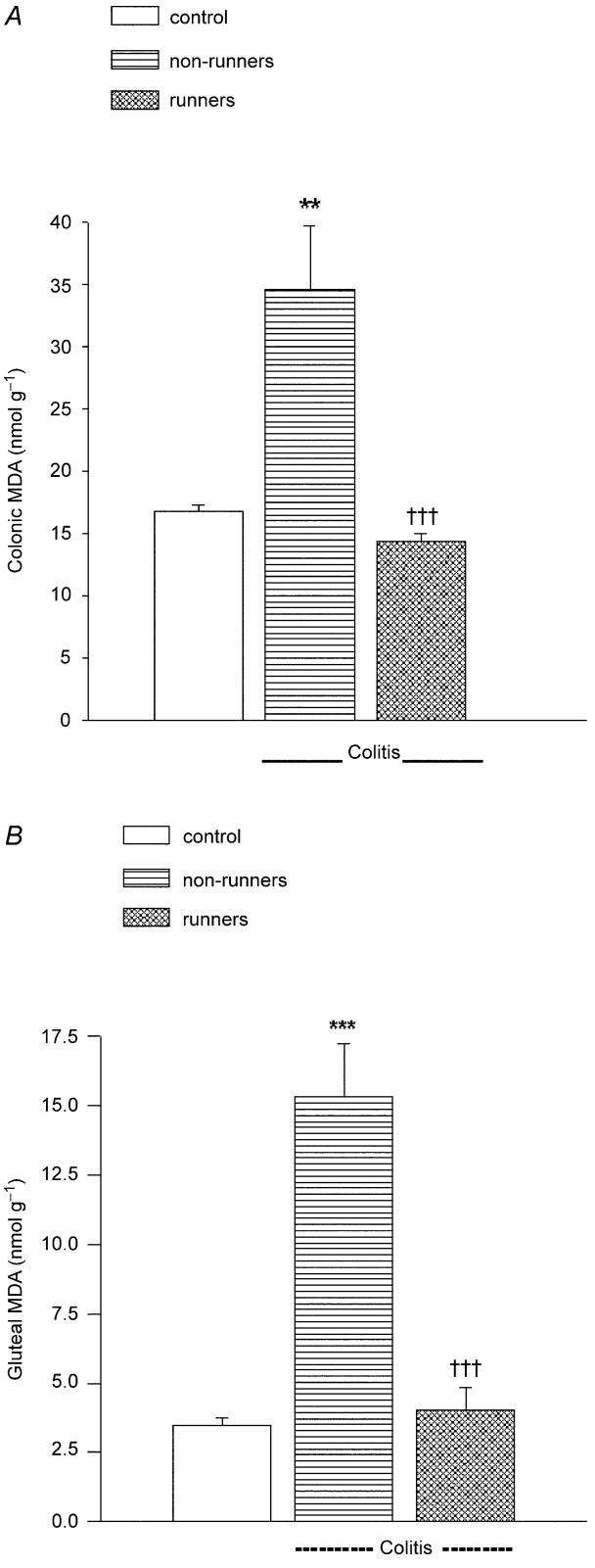

Figure 4. Malondialdehyde (MDA) levels of colonic (*A*) and gluteus muscle tissues (*B*)
**$P < 0.01$, ***$P < 0.001$ compared to control group; †††$P < 0.001$ compared to corresponding non-runner group.

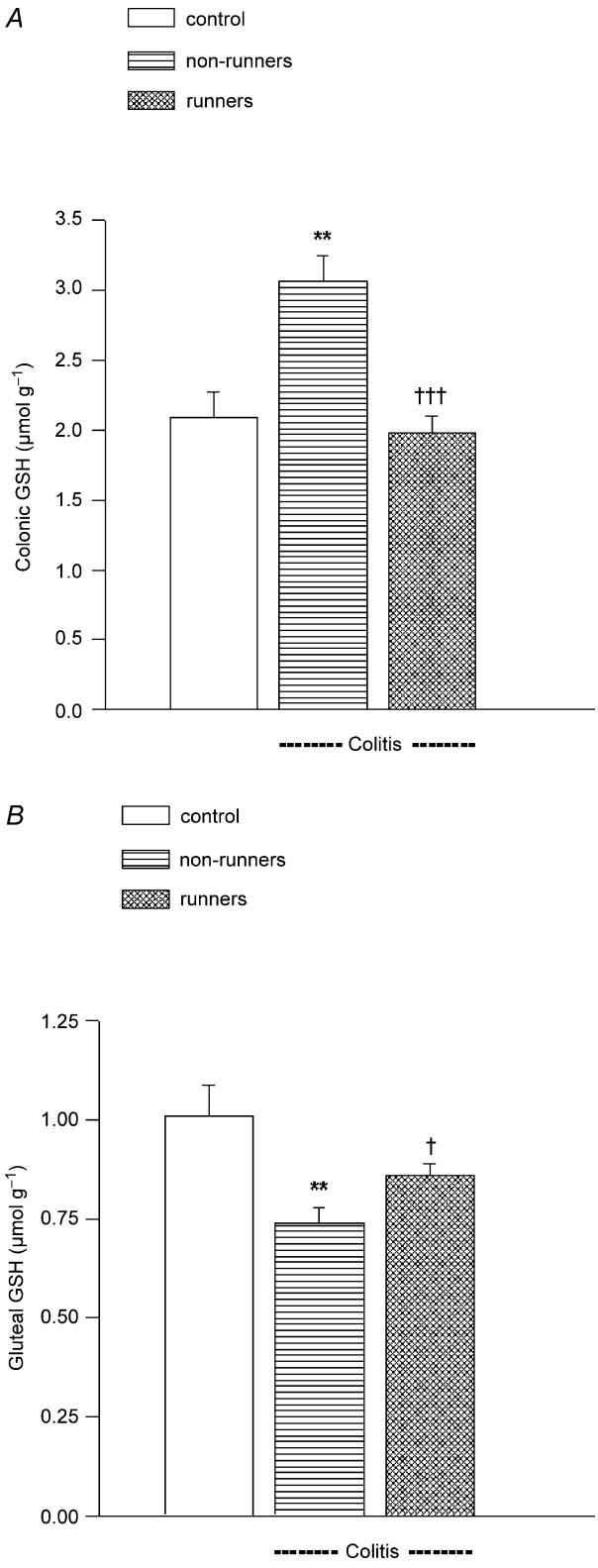

Figure 5. Gluthatione (GSH) levels of colonic (*A*) and gluteus muscle tissues (*B*)
$^{**}P < 0.01$ compared to control group; †$P < 0.05$, †††$P < 0.001$ compared to corresponding non-runner group.

In the sedentary colitis group, histological analysis revealed the presence of a massive loss of surface epithelium, degeneration of crypts and denudation of lamina propria, severe submucosal oedema, inflammatory cell infiltration and vasculitis (Fig. 6). In contrast, mild degeneration of the epithelium with normal crypts, moderate inflammatory cell infiltration and vasculitis were observed in the runner rats with colitis. No histological difference was observed in the muscle tissues of the experimental groups.

Discussion

The results of the present study demonstrate that physical activity attenuates the severity of acetic acid-induced colonic inflammation by a neutrophil-dependent mechanism, suggesting a potent anti-inflammatory effect of low-intensity exercise on the oxidative injury of the colon. Moreover, colitis-induced oxidative injury of the skeletal muscle is also ameliorated by regular exercise.

The occurrence of gastrointestinal symptoms, such as heartburn, nausea, vomiting, abdominal cramps, diarrhoea and gastrointestinal bleeding, is common during vigorous sports, causing the athlete to limit exercise performance by reducing exercise intensity or duration (Rehrer *et al.* 1989; Brouns & Beckers, 1993; Peters *et al.* 2001; Moses, 2005). Although the mechanisms by which exercise causes gastrointestinal symptoms are not well known, reduced gastrointestinal blood flow, increased gastrointestinal motility and mechanical bouncing, and alterations in neuroendocrine modulation have been suggested to play roles. In contrast, several studies indicate an inverse relationship between physical activity and the risk of gastrointestinal-related diseases, such as colonic cancer, cholelithiasis, diverticular disease or constipation (Peters *et al.* 2001). The primary postulated mechanisms include reduced intestinal transit time and enhanced activity of the free radical scavenging enzymes (Shephard & Shek, 1998). Accordingly, an inverse correlation was observed between the symptoms of irritable bowel syndrome and exercise (Colwell *et al.* 1998). However, inconsistent results have been obtained regarding the effect of physical activity on ulcerative colitis or Crohn's disease (Sorensen *et al.* 1987; Klein *et al.* 1998; Lee *et al.* 2005). The results of the present study demonstrate that regular running exercise in rats for 6 weeks reduces the severity of colitis-induced colonic damage by attenuating the oxidative tissue damage and enhancing the antioxidative enzyme activity.

It has been proposed that oxygen-derived free radicals are implicated in the pathogenesis of IBD (Simmonds *et al.* 1992; Simmonds & Rampton, 1993), while free radical-mediated lipid peroxidation is an important contributing factor in the development of acetic acid-mediated colonic

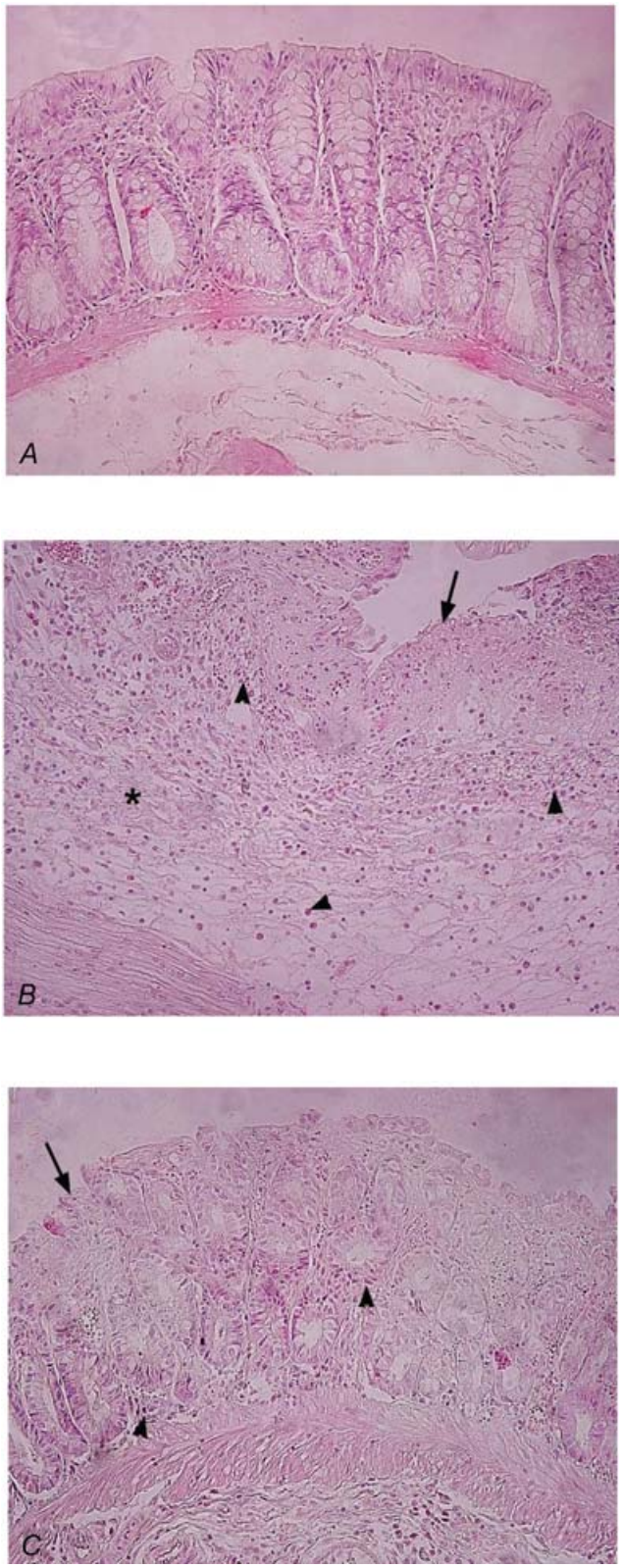

Figure 6. Micrographs showing the histopathological analysis of the colonic tissue
Control group (*A*), illustrating regular colon mucosa and submucosa. Non-runner colitis group (*B*), showing massive loss of surface epithelium (arrow), severe submucosal oedema (*) and inflammatory cell infiltration (arrowheads). Runner colitis group (*C*), showing mild degeneration of surface epithelium (arrow) with regular colonic glands and mild inflammatory cell infiltration (arrowheads). H&E staining. original magnifications: ×200.

damage (Xia *et al.* 1996). Several reports have shown that the formation of malondialdehyde, an end product of lipid peroxidation, is increased in the colonic tissue with inflammation (Yoshida *et al.* 1999; Mahgoub *et al.* 2003; Iseri *et al.* 2005). In accordance with the previous results, in the present study, acetic acid-induced colitis resulted in increased production of MDA in the colon. In contrast, regular exercise prior to colitis induction suppressed colonic MDA production, suggesting that exercise-induced preconditioning reduces lipid peroxidation and thereby supports the maintenance of cellular integrity. Interestingly, MDA production in the skeletal muscle was also elevated following colitis induction. Since acetic acid-induced colitis initiates a systemic inflammatory response, it appears that preconditioning through repetitive exercise provides protection not only for the colon but also for the remote targets of the inflammatory challenge.

Exercise is one of the physiological conditions characterized by increased production of free radicals (Clarkson, 1995). The production of free radicals increases in parallel with the increase in oxygen consumption during exercise, and this increase is directly related to the intensity and/or the duration of exercise (Ji, 1996). In contrast, the antioxidant enzymes, which constitute a defense mechanism against free radicals produced during exercise, are also affected by the exercise (Clarkson, 1995). Although much contradiction exists, it is generally accepted that regular physical activity leads to an increase in the activities of antioxidant enzymes, especially in muscles (Ji, 1993, 1996; Lawler & Powers, 1998). As well as the changes in exercising muscle, it was recently shown that exercise preconditioning inhibits inflammatory brain damage and decreases the expression of inflammatory mediators (Ding *et al.* 2005). It was reported that the protective effect of exercise might be due in part to suppression of the inflammatory process through reduced plasma levels of chemokines (Troseid *et al.* 2004).

One of the major constituents of intracellular protective mechanisms is glutathione, which provides protection against various noxious stimuli, including oxidative stress. Glutathione, as the main component of the endogenous non-protein sulfhydryl pool, is capable of repairing membrane lipid peroxides by interacting with free radicals to yield more stable elements (Ross, 1988; Shaw *et al.* 1990). Reduced GSH is known to be a major low molecular weight scavenger of free radicals in the cytoplasm (Shaw *et al.* 1990), limiting the propagation of free-radical reactions. Our results support the fact that depletion of colonic GSH is one of the major factors permitting lipid peroxidation

and subsequent colonic damage. Since increased physical activity for 6 weeks prevented colonic GSH depletion, it appears that exercise supports the maintenance of antioxidant capacity in protecting the colonic tissue against oxidative stress. In accordance with the alterations in colonic GSH stores, colitis led to reduction in the GSH content of the skeletal muscle, supporting the generalized effect of colonic inflammation on the antioxidative status of the other tissues. Increased physical activity, in contrast, preserved the GSH content to protect the skeletal muscle against the systemic inflammatory response. In accordance with the present findings, Gunduz *et al.* (2004) have shown that long-term regular exercise increases the antioxidant capacity in rats. Furthermore, regular exercise attenuated lymphocyte apoptosis induced by oxidative stress, possibly by improving intracellular antioxidative capacity (Wang & Huang, 2005). In obese Zucker rats, exercise reversed the decreased enzyme activities of hepatic superoxide dismutase (SOD), GSH and glutathione peroxidase (Chang *et al.* 2004). Similarly, exercise training reduced oxidative stress via mechanisms that increased SOD activity in spontaneously hypertensive rats (Kohno *et al.* 2002).

It has been previously reported that active lesions in ulcerative colitis involve the migration of activated neutrophils and macrophages (Shiratora *et al.* 1989; Simmonds & Rampton, 1993). The tissue-associated MPO, which is known to provide an index of neutrophil infiltration, plays a fundamental role in oxidant production by neutrophils (Weiss & Ward, 1982). Exercise promotes the release of neutrophils into the circulation, and neutrophil activation during exercise may challenge endogenous antioxidant defence mechanisms (Peake & Suzuki, 2004). Our observations of elevated MPO levels in colonic tissues indicate that neutrophil accumulation contributes to the colitis-induced oxidative injury and that exercise appears to have a preventive effect through the inhibition of neutrophil infiltration. Exercise training in patients with claudication was shown to decrease neutrophil activation and degranulation and thereby reduce the inflammation (Turton *et al.* 2002). In principle, improved responsiveness of neutrophils to exercise of moderate intensity could mean that individuals participating in exercise may have improved resistance to infection (Peake, 2002). Conversely, competitive athletes undertaking regular intense exercise may be at greater risk of infections. In addition to exercise-induced reduction in colonic neutrophil accumulation, a similar suppression of neutrophils was evident in the muscle tissue accustomed to regular exercise.

Exercise-induced increases in aerobic fitness were shown to have beneficial short-term and long-term effects on psychological outcomes (DiLorenzo *et al.* 1999). Among the possible explanations for psychological outcomes are the direct effects of neurotransmitters (e.g. serotonin) in the brain that function to elevate mood (McDonald & Hodgdon, 1991; LaFontaine *et al.* 1992). The present data demonstrate that regular exercise attenuates the anxiety of the animals stressed when placed on the holeboards. However, in accordance with previous studies demonstrating that psychological stress might amplify intestinal inflammation (Collins, 2001), the severity of the colitis was enhanced as the level of anxiety was exaggerated. In contrast, the degree of anxiety was reduced in regularly exercised rats with significant attenuation of colonic inflammation. Similarly, physical activity was postulated to reduce disease activity and perceived stress in IBD patients, while general well-being and quality of life were improved (Loudon *et al.* 1999). Future research needs to examine the psychological benefits of exercise on colitis pathogenesis in non-clinical and clinical settings, to explain the correlation between the severity of colonic inflammation and the exercise intensity and duration.

In conclusion, this study implicates the importance of exercise as a non-pharmacological intervention in controlling the course of inflammatory bowel diseases, specifically during stressful conditions that trigger the exacerbation of the symptoms.

References

Aykaç G, Uysal M, Yalçın AS, Koçak-Toker N, Sivas A & Öz H (1985). The effect of chronic ethanol ingestion on hepatic lipid peroxidase and glutathione transferase in rats. *Toxicology* **36**, 71–76.

Babbs CF (1992). Oxygen radicals in ulcerative colitis. *Free Radic Biol Med* **13**, 169–181.

Bi L & Triadafilopoulos G (2003). Exercise and gastrointestinal function and disease: an evidence-based review of risks and benefits. *Clin Gastroenterol Hepatol* **1**, 345–355.

Blair SN, Kannel WB, Kohl HW, Goodyear N & Wilson PWF (1989). Surrogate measures of physical activity and physical fitness: evidence for sedentary traits of resting tachycardia, obesity, and low vital capacity. *Am J Epidemiol* **129**, 1145–1156.

Boissier JR & Simon P (1962). La reaction d'exploration chez la souris. *Thérapie* **17**, 1225–1232.

Bradley PP, Priebat DA, Christersen RD & Rothstein G (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Invest Dermatol* **78**, 206–209.

Brouns F & Beckers E (1993). Is the gut an athletic organ? Digestion, absorption and exercise. *Sports Med* **15**, 242–257.

Byrne A & Byrne DG (1993). The effects of exercise on depression, anxiety and other mood states: a review. *J Psychosom Res* **37**, 565–574.

Casini A, Ferrali M, Pompella AS, Maellaro E & Comporti M (1986). Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene toxicated mice. *Am J Pathol* **123**, 520–531.

Chang SP, Chen YH, Chang WC, Liu IM & Cheng JT (2004). Increase of anti-oxidation by exercise in the liver of obese Zucker rats. *Clin Exp Pharmacol Physiol* **31**, 506–511.

Clarkson PM (1995). Antioxidants and physical performance. *Crit Res Food Sci Nutr* **35**, 131–141.

Collins SM (2001). Stress and the Gastrointestinal Tract IV. Modulation of intestinal inflammation by stress: basic mechanisms and the clinical relevance. *Am J Physiol Gastrointest Liver Physiol* **280**, G315–G318.

Colwell LJ, Prather CM, Phillips SF & Zinsmeister AR (1998). Effects of an irritable bowel syndrome educational class on health-promoting behaviors and symptoms. *Am J Gastroenterol* **93**, 901–905.

DiLorenzo TM, Bargman EP, Stucky-Ropp R, Brassington GS, Frensch PA & LaFontaine T (1999). Long-term effects of aerobic exercise on psychological outcomes. *Prev Med* **28**, 75–85.

Ding YH, Young CN, Luan X, Li J, Rafols JA, Clark JC, McAllister JP 2nd & Ding Y (2005). Exercise preconditioning ameliorates inflammatory injury in ischemic rats during reperfusion. *Acta Neuropathol* **109**, 237–246.

Erkanli K, Kayalar N, Erkanli G, Ercan F, Sener G & Kirali K (2005). Melatonin protects against ischemia/reperfusion injury in skeletal muscle. *J Pineal Res* **39**, 238–242.

File SE & Wardill AG (1975). Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia* **44**, 53–59.

Fletcher GF, Blair SN, Blumenthal J, Casperson C, Chaitman B, Epstein L, Fall H, Froelicher ES, Froelicher VF & Pina IL (1992). Statement of exercise. Benefits and recommendations for physical activity programs for all Americans. *Circulation* **86**, 340–344.

Gue M, Bonbonne C, Fioramonti J, More J, Del Rio-Lacheze L, Comera C & Bueno L (1997). Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved. *Am J Physiol* **272**, G84–G91.

Gulpinar MA, Ozbeyli D, Arbak S & Yegen BC (2004). Anti-inflammatory effect of acute stress on experimental colitis is mediated by cholecystokinin-B receptors. *Life Sci* **75**, 77–91.

Gunduz F, Senturk UK, Kuru O, Aktekin B & Aktekin MR (2004). The effect of one year's swimming exercise on oxidant stress and antioxidant capacity in aged rats. *Physiol Res* **53**, 171–176.

Iseri SO, Sener G, Saglam B, Gedik N, Ercan F & Yegen BC (2005). Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. *Peptides* **26**, 483–491.

Ji LL (1993). Antioxidant enzyme response to exercise and ageing. *Med Sci Sports Exerc* **25**, 225–231.

Ji LL (1996). Exercise, oxidative stress, and antioxidants. *Am J Sports Med* **24**, S20–S24.

Klein I, Reif S, Farbstein H, Halak A & Gilat T (1998). Preillness non dietary factors and habits in inflammatory bowel disease. *Ital J Gastroenterol Hepatol* **30**, 247–251.

Kohno H, Furukawa S, Naito H, Minamitani K, Ohmori D & Yamakura F (2002). Contribution of nitric oxide, angiotensin II and superoxide dismutase to exercise-induced attenuation of blood pressure elevation in spontaneously hypertensive rats. *Jpn Heart J* **43**, 25–34.

LaFontaine TP, DiLorenzo TM, Frensch PA, Stucky-Ropp RC, Bargman EP & McDonald DG (1992). Aerobic exercise and mood. A brief review, 1985–90. *Sports Med* **13**, 160–170.

Lawler JM & Powers SK (1998). Oxidative stress, antioxidant status, and the contracting diaphragm. *Can J Appl Physiol* **23**, 23–55.

Lee N, Radford-Smith G & Taaffe DR (2005). Bone loss in Crohn's disease: exercise as a potential countermeasure. *Inflamm Bowel Dis* **11**, 1108–1118.

Loudon CP, Corroll V, Butcher J, Rawsthorne P & Bernstein CN (1999). The effects of physical exercise on patients with Crohn's disease. *Am J Gastroenterol* **94**, 697–703.

McDonald DG & Hodgdon JA (1991). *Psychological Effects of Aerobic Fitness Training: Research and Therapy*. Springer-Verlag, New York.

MacPherson BR & Pfeiffer CJ (1978). Experimental production of difuse colitis in rats. *Digestion* **17**, 135–150.

Mahgoub AA, El-Medany AA, Hager HH, Mustafa AA & El-Sabah DM (2003). Evaluating the prophylactic potential of zafirlukast against the toxic effects of acetic acid on the rat colon. *Toxicol Lett* **145**, 79–87.

Marco EM, Llorente R, Pérez-Álvarez L, Moreno E, Guaza C & Viveros MP (2005). The κ-opioid receptor is involved in the stimulating effect of nicotine on adrenocortical activity but not in nicotine induced anxiety. *Behav Brain Res* **163**, 212–218.

Maunder RG (2005). Evidence that stress contributes to inflammatory bowel disease: evaluation, synthesis, and future directions. *Inflamm Bowel Dis* **11**, 600–608.

Mitchell TW, Turner N, Hulbert AJ, Else PL, Hawley JA, Lee JS, Bruce CR & Blanksby SJ (2004). Exercise alters the profile of phospholipid molecular species in rat skeletal muscle. *J Appl Physiol* **97**, 1823–1829.

Moses FM (2005). Exercise-associated intestinal ischemia. *Curr Sports Med Rep* **4**, 91–95.

Peake JM (2002). Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. *Exerc Immunol Rev* **8**, 49–100.

Peake J & Suzuki K (2004). Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. *Exerc Immunol Rev* **10**, 129–141.

Peters HP, De Vries WR, Vanberge-Henegouwen GP & Akkermans LM (2001). Potential benefits and hazards of physical activity and exercise on the gastrointestinal tract. *Gut* **48**, 435–439.

Rehrer NJ, Janssen GM, Brouns F & Saris WH (1989). Fluid intake and gastrointestinal problems in runners competing in a 25-km race and a marathon. *Int J Sports Med* **10**, S22–S25.

Ross D (1988). Glutathione, free radicals and chemotherapeutic agents. *Pharmacol Ther* **37**, 231–249.

Sanahan F (1993). Pathogenesis of ulcerative colitis. *Lancet* **342**, 407–411.

Shaw S, Herbert V, Colman N & Jayatilleke E (1990). Effect of ethanol-generated free radicals on gastric intrinsic factor and glutathione. *Alcohol* **7**, 153–157.

Shephard RJ & Shek PN (1998). Associations between physical activity and susceptibility to cancer: possible mechanisms. *Sports Med* **26**, 293–315.

Shiratora Y, Aoki S, Takada H, Kiriyama H, Ohto K, Hai K, Teraoka H, Matano S, Matsumoto K & Kamii K (1989). Oxygen-derived free radical generating capacity of polymorphonuclear cells in patients with ulcerative colitis. *Digestion* **44**, 163–171.

Simmonds NJ, Allen RE, Stevens TR, Van Someren RN, Blake DR & Rampton DS (1992). Chemiluminescence assay of mucosal reactive oxygen metabolites in inflammatory bowel disease. *Gastroenterology* **103**, 186–196.

Simmonds NJ & Rampton DS (1993). Inflammatory bowel disease – a radical view. *Gut* **34**, 865–868.

Simren M (2002). Physical activity and the gastrointestinal tract. *Eur J Gastroenterol Hepatol* **14**, 1053–1056.

Sonnenberg A (1990). Occupational distribution of inflammatory bowel disease among German employees. *Gut* **31**, 1037–1040.

Sorensen VZ, Olsen BG & Binder V (1987). Life prospects and quality of life in patients with Crohn's disease. *Gut* **28**, 382–385.

Troseid M, Lappegard KT, Claudi T, Damas JK, Morkrid L, Brendberg R & Mollnes TE (2004). Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. *Eur Heart J* **25**, 349–355.

Turton EPL, Coughlin PA, Kester RC & Scott DJA (2002). Exercise training reduces the acute inflammatory response associated with claudication. *Eur J Vasc Surg* **23**, 309–316.

Wallace JL, Keenan CM, Gale D & Shoupe TS (1992). Exacerbation of experimental colitis by nonsteroidal anti-inflammatory drugs is not related to elevated leukotriene B_4 synthesis. *Gastroenterology* **102**, 18–27.

Wang JS & Huang YH (2005). Effects of exercise intensity on lymphocyte apoptosis induced by oxidative stress in men. *Eur J Appl Physiol* **12**, 1–8.

Weiss SJ & Ward PA (1982). Immune complex-induced generation of oxygen metabolites by human neutrophils. *J Immunol* **129**, 309–319.

Xia B, Deng CS, Chen DJ, Zhou Y & Xiao JQ (1996). Role of copper zinc superoxide dismutase in the short-term treatment of acetic acid-induced colitis in rats. *Acta Gastroenterol Latinoam* **26**, 227–230.

Yoshida N, Yoshikawa T, Yamaguchi T, Naito Y, Tanigawa T, Murase H & Kondo M (1999). A novel water-soluble vitamin E derivative protects against experimental colitis in rats. *Antioxid Redox Signal* **1**, 555–562.

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