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Repeated bouts of eccentrically biased endurance exercise stimulate salivary IgA secretion rate

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ABSTRACT: To determine the salivary secretory immunoglobulin A (sIgA) response to repeated bouts of unaccustomed, downhill running (eccentrically biased) and examine potential protective immunological adaption from a repeated bout effect. Eleven active but untrained males (age: 19.7±0.4 years; VO_{2peak}: 47.8± 3.6 ml·kg^{-1}·min $^{-1}$) performed two 60 min bouts (Run 1 and Run 2) of downhill running (-13.5% gradient), separated by 14 days, at a speed eliciting 75% of their VO_{2peak} on a level grade. Saliva samples were collected before (baseline), immediately post exercise (IPE), and every hour for 12 h and every 24 h for 6 days after each run. Salivary sIgA concentration was measured and sIgA secretion rate was calculated. Results were analysed using repeated measures ANOVA (12 h period: 2x14; 24 h intervals: 2x7; $p \leq 0.05$) with Tukey post-hoc tests where appropriate. Results are reported as means ± SE. There was a significant ($p < 0.0001$) interaction effect for sIgA secretion rate, IPE, with higher values after Run 2, as well as a significant ($p < 0.01$) time effect with elevated levels IPE and between 24 h and 144 h. There was a run effect ($p < 0.0001$), with the sIgA secretion rate significantly higher after Run 2. Repeated bouts of unaccustomed, eccentrically biased exercise induced alterations in the salivary sIgA secretion rate. This may serve as a protective mucosal adaptation to exercise-induced tissue damage.

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INTRODUCTION

Eccentrically biased exercise (e.g. downhill running) and the associated muscle adaptations may have important clinical and rehabilitation implications. Eccentric training has been shown to reduce the incidence of skeletal muscle tears, rehabilitate tendinopathies, prevent atrophy in the aged [1] and reduce cardiovascular as well as metabolic disease risk factors and markers [2]. Also, eccentric training has been recommended for patients enrolled in cardiac rehabilitation because of the reduced metabolic cost of performing this mode of exercise [3]. It has been shown that there are marked reductions (-20% to -30%) in oxygen consumption and energy expenditure when running downhill at a given speed compared with running on a level grade [4, 5]. In regard to immune function, research has examined leukocytes and pro- and anti-inflammatory cytokine responses to single and repeated bouts of eccentrically biased exercise [6, 7].

It is well established that an unaccustomed bout of eccentrically biased exercise induces trauma to muscle/connective tissue [8]. Furthermore, it has been verified that when a similar bout of exercise is performed within a few days, until approximately six weeks after this initial bout, there is significantly less damage [9-11]. The reduced tissue damage seen after the second bout of eccentrically biased exercise is referred to as the "repeated bout effect" [12]. This effect is associated with reductions in indirect markers of muscle damage, such as strength loss, levels of muscle soreness, serum levels of muscle enzymes such as creatine kinase [9], leukocytes [13], pro-inflammatory cytokines and increases in anti-inflammatory cytokines [7].

There has been limited research examining the effect of eccentric exercise on humoral immunity (HI), specifically the immunoglobulin/antibody response. Smith hypothesised that exercise-induced tissue damage may alter HI including immunoglobulin (Ig)/antibody responses in a negative way that could increase the risk of viral infections and allergy [14]. In support of this, Mckune et al. demonstrated that systemic Ig isotypes and subclasses responded differently to repeated bouts of eccentrically biased exercise [15]. In addition, Edwards et al. demonstrated that a single bout of eccentric exercise enhances the circulating antibody response to the influenza virus [16]. These authors suggested that stressed cells (e.g. damaged myocytes) may release danger signals which activate dendritic

cells [17]. Shi and Rock found that damaged cells in vivo stimulated antigen-presenting dendritic cells to acquire antigen and/or mature and migrate to lymph nodes [18]. Thus, muscle cells stressed by eccentric exercise may release signals which could enhance the HI antibody response to a vaccination [16]. To date, the mucosal immunoglobulin/antibody (e.g. salivary secretory IgA (sIgA)) response to a bout or repeated bouts of eccentrically biased exercise has not been examined. Understanding the effect of this type of exercise modality on sIgA would be useful in light of previous research demonstrating that strenuous endurance training, predominantly concentrically biased (e.g. swimming or running on a level or uphill gradient) is associated with a decrease in sIgA as well as an increased risk of upper respiratory tract infections in athletes [25, 26].

sIgA is the major Ig present in the secretions of the mucosa, such as saliva, bronchial fluid, gastrointestinal, vaginal and nasal secretions, as well as in tears and breast milk [19]. sIgA has an important role in primary protection against bacterial, viral and protozoal infections of the mucosa [20]. Therefore, understanding the factors that regulate its production and movement into the upper airways is important for reducing the risk of illness in athletes [25]. IgA is produced by long-lived plasma B cells [19, 21] that are under the influence of T-cell-generated cytokines. These cytokines play a critical role in regulating the amount of secretory sIgA that appears in the saliva [21]. Normally, T cells maintain high levels of the T helper 2 cell IgA stimulating cytokines (IL-4, IL-5, IL-6 and IL-10), the T helper 17 cytokine, IL-21 [22], and most importantly the T helper 3 cell anti-inflammatory cytokine, TGF-β [23]. These are counterbalanced by T helper 1 cell IgA inhibiting cytokines such as IFN-γ [21,24]. TGF-β, IL-21 and the T helper 2 cytokines are responsible for the activation, differentiation and proliferation of plasma B cells into IgA Ig/antibody secreting cells [21,22,23]. Secretion of IgA into saliva requires specialized transport of dimeric IgA from the circulation across the mucosal epithelial barrier, since tight intercellular junctions prevent the diffusion of large molecules between epithelial cells [25,26]. The pro-inflammatory cytokines TNF-β and IL-1 β play an important role in regulating the transport of dimeric IgA into the mucosa through increasing the polymeric immunoglobulin receptor (pIgR) [27]. This receptor specifically transports IgA across the epithelium via transcytosis after dimeric IgA, produced by plasma cells, binds to the pIgR molecule expressed on the basolateral surface of the epithelium [28]. Cleavage of this molecule on the luminal side of the epithelium releases sIgA into the airway [27].

Although there is currently no single supported theory to explain the repeated-bout effect, it is clear that there is interaction between the levels of muscle damage and the immune/inflammatory response following eccentrically biased exercise [7,14,16] and it seems that the co-ordination of these responses may contribute to the previously demonstrated adaptive response to repeated bouts of similar exercise [29].

Considering that there is an immune response to eccentrically biased exercise that includes alterations in systemic pro- and anti-inflammatory cytokines [7], some of which are responsible for sIgA production, the aim of the present study was to investigate the salivary secretory IgA (sIgA) secretion rate in response to two bouts of downhill running. Based on the previous work of Edwards et al., who demonstrated that eccentrically biased exercise enhanced circulating antibody levels, and our own work where repeated bouts of downhill running altered cytokines [7] and Igs [15], we hypothesised that a repeated bout of downhill running would enhance the sIgA secretion rate.

MATERIALS AND METHODS

Participants. Eleven healthy, active but untrained Caucasian males were recruited to participate in the study. Initial selection criteria included the following: age 18-30 years; non-smokers; no history of leg injury or any other medical condition that would be exacerbated by a bout of downhill running; no regular use of any anti-inflammatory medication or anti-oxidants. Participants read and signed an informed consent form, which had been approved by the Institution's Ethics Committee.

Assessment of $\dot{V}O_2$peak and Determination of Running Speeds

Ten days before the first downhill run a standard Bruce treadmill (Quinton Instrument Co. Seattle, Washington) protocol was used to determine the $\dot{V}O_2$peak of each participant. Continuous respiratory measurements were recorded using a MedGraphics CardiO2 combined $\dot{V}O_2$/ECG exercise system (Medical Graphics Corporation Chicago, Illinois). Heart rate was recorded at the end of each minute using a Polar TM Heart Rate Monitor, and ratings of perceived exertion (RPE) were recorded at the end of each stage (every 3 minutes) as well as when participants reached volitional exhaustion. The test was accepted as $\dot{V}O_2$peak if two of the following criteria were attained: Respiratory Exchange Ratio $\geq$ 1.1, *and/or* RPE $\geq$ 19 on the 15-point RPE Scale, *and/or* maximum heart rate (HR_{max}) within ±20 beats of age predicted HR_{max}. Seventy-five percent of $\dot{V}O_2$peak was calculated and a metabolic equation was used to determine the speed, on a level grade, that would elicit this $\dot{V}O_2$ [7]. This treadmill speed was the designated speed for each participant for the downhill run.

Repeated Downhill Run Protocol

This study formed part of a published study that reported on the responses of indirect markers of muscle damage to two bouts of downhill running [30]. Serum creatine kinase and delayed onset muscle soreness were significantly reduced after the second bout of running, thus demonstrating a repeated bout effect [30].

Participants were instructed to ingest a normal mixed diet, to be well hydrated, and to refrain from any strenuous physical activity for at least 7 days prior to each run. Each participant performed two identical bouts of downhill running, spaced 14 days apart (Run 1 and Run 2). The runs on both days occurred between 5:00 and 11:00 am and at approximately the same time for each participant. The participants performed the runs in a fasted state (at least 8 hours

post-absorption). At the start of the run, participants warmed up for 5 minutes by running on a level grade at the pre-determined speed. The treadmill was then lowered to a -13.5% decline and participants ran for 60 minutes [30]. The participants remained in the exercise testing laboratory (ETL) for 12 h after the run. They were provided with food and fluid and encouraged to eat, and especially to drink water, ad libitum. However, they were requested to adhere to the saliva sampling precautions relating to eating and drinking as outlined below.

Saliva Sampling, Handling and Preparation

Saliva samples were collected at the following times: pre-exercise (PRE), immediately post exercise (IPE), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h post-run. In addition, participants were required to return in a fasted state to the ETL at 24, 48, 72, 96, 120 and 144 h after each run for an additional saliva collection on each day. Upon arriving at the ETL, participants sat quietly for fifteen minutes as a standardised saliva collection procedure was followed [31]. Saliva samples were collected via unstimulated passive drool over a time period of five minutes. Whilst seated, the participants were asked to lean slightly forward, tilt their heads down and accumulate saliva in the floor of the mouth for a minute, which was subsequently swallowed. Following this there was a four minute collection where saliva was dribbled through a 5 cm plastic straw into a pre-weighed polypropylene cryovial (5 ml capacity). Care was taken to allow saliva to dribble into the collecting tubes with minimal orofacial movement. The participants were also requested to 1) refrain from brushing their teeth prior to each of the saliva collections to eliminate possible contamination of samples due to bleeding gums, 2) avoid dairy products for 20 minutes before each sample collection, 3) avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection (these have all been shown to impact on the saliva pH, altering assay results), 4) rinse their mouths with water and swallow to remove food residue before sample collection and increase hydration, and 5) wait at least 10 minutes after rinsing before collecting saliva to avoid sample dilution. Following the collection the cryovial was re-weighed. The saliva samples were then centrifuged at 3000 rpm for 15 minutes to remove mucins and the supernatant was stored frozen at –80°C until assay.

Salivary Secretory IgA Analysis

Salivary sIgA (μg IgA · min^{-1}) concentration was determined using an indirect enzyme immunoassay kit (Salimetrics, State College, USA). The concentration of sIgA was expressed as the salivary sIgA secretion rate (s-IgA) (μg sIgA · min^{-1}), or the total amount of sIgA appearing on the mucosal surface per time unit [32]. sIgA secretion rate was calculated by multiplying absolute sIgA concentration by saliva flow rate (ml · min^{-1}). Saliva flow rate was calculated by dividing the total amount of saliva obtained in each sample (ml) (post-collection cryovial weight – pre-collection cryovial weight) by the time taken to produce the sample (4 minutes) [32].

Statistical Analysis

A repeated measures analysis of variance (ANOVA) was used to analyse the main effects (run and time) and the interaction effects (differences between Run 1 and Run 2 at any time point(s)) for salivary sIgA. To determine whether changes occurred during the 12 h following downhill running or during the subsequent 24 h periods, data were analysed separately (12 h period: 2x14; 24 h intervals: 2x7; $p \leq 0.05$) with Tukey post-hoc tests where appropriate.

RESULTS

Physical Characteristics of Participants. Physical characteristics (mean ± SD) were: age (years) = 19.7 ± 0.4; height (m) = 1.79 ± 4.3; mass (kg) = 78.5 ± 3.0; body fat (%) = 14.6 ± 3.2; $\dot{V}O_2$ peak (ml · kg^{-1} · min^{-1}) = 47.8 ± 3.6.

Salivary Secretory IgA Secretion Rate

The primary question in this study was whether the participants' sIgA secretion rate differed between the two bouts of downhill running. There was a significant ($p < 0.0001$) interaction effect for sIgA secretion rate, IPE, with values 3.5-fold higher after Run 2 (851 ± 70.85 μg · min^{-1}) compared to Run 1 (245 ± 60.85 · min^{-1}). There was a significant ($p < 0.0001$) run effect with sIgA secretion rate significantly higher (+30%) after Run 2 (556 ± 15.8 · min^{-1}) compared to Run 1 (428 ± 14.8 · min^{-1}). There was a significant ($p < 0.01$) time effect with sIgA elevated IPE (+113%) and from 24 h to 144 h (+118% to 164%) compared to pre-exercise levels (Figure 1).

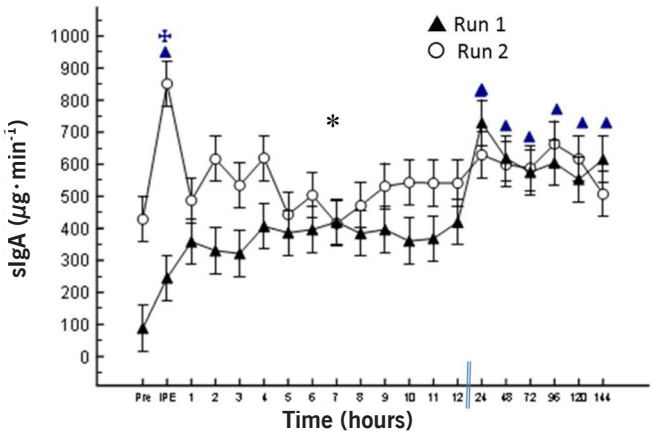

FIG. 1. Salivary sIgA secretion rate levels (means ± SE) before and after Run 1 and Run 2.
Note: ✠ = interaction effect with the IPE level after Run 2 significantly ($p < 0.0001$) higher than after Run 1.
* = run effect with Run 2 significantly ($p < 0.0001$) higher than Run 1.
▲ = significant ($p < 0.01$) time effect with an elevation IPE, and at 24-144 h after both runs compared to baseline values. Parallel forward slashes between "12 h" and "24 h" denote a change in time interval.

DISCUSSION

This is the first study to compare changes in salivary sIgA secretion rate in response to repeated bouts of unaccustomed, eccentrically biased endurance exercise. The novel finding of an increased sIgA secretion rate after the second bout of downhill running, supported by the significant interaction and run effects, as well as the demonstrated elevation in sIgA secretion rate IPE compared to baseline values, is indicative of a repeated bout effect. The significant increase in sIgA IPE also concurs with previous findings of increases in sIgA following moderate intensity exercise or training [33].

The results suggest that a repeated bout effect may extend to mucosal immunity in the upper respiratory tract. Currently the mechanisms regulating the increase in sIgA secretion rate with concentrically biased moderate intensity exercise and training [33] remains speculative, although an exercise-related increase in sympathetic nervous system activation may be involved [34]. Likewise, the mechanism regulating the increase in sIgA secretion rate that occurred in the present study, in response to a bout and a repeated bout of eccentrically biased exercise (i.e. downhill running), is speculative. We propose that either, or a combination, of the following three mechanisms may have been involved in elevating the sIgA secretion rate, in addition to a possible influence of the sympathetic nervous system [34].

Anti-inflammatory Cytokine Mechanism

sIgA is traditionally regarded as a non-inflammatory antibody [35] and its synthesis is regulated by the T helper 3 cell anti-inflammatory cytokine TGF-b [23], with the T helper 17 cell cytokine IL-21, and T helper 2 cell cytokines IL-4, IL-5, IL-6 and IL-10, acting as adjunct cytokines promoting sIgA-secreting B cell proliferation and differentiation [22, 21]. Moderate exercise has been reported to enhance anti-inflammatory mechanisms, including increasing circulating levels of TGF-β [23], while eccentrically biased endurance exercise has been shown to elevate circulating IL-6 and IL-10 [7]. In addition, circulating IL-10 has been shown to increase by 95% after a second bout of eccentrically biased endurance exercise [7]. Therefore, alterations in the levels of these cytokines with exercise and/or exercise-induced muscle damage may be responsible for stimulating sIgA synthesis and appearance in the mucosa through their effects on plasma B cells [21].

Memory B Cell Mechanism

A second mechanism may be related to the local release of inflammatory mediators by damaged skeletal muscle/tissue. These mediators have been shown to activate dendritic cells and enhance their migration to the lymph nodes, as well as their antigen-presenting capabilities [18]. The result of this enhanced antigen presentation could be an increased primary immunoglobulin/antibody response by plasma B cells [16] resulting in an enhanced sIgA secretion rate. Certain plasma B cells become memory B cells and are responsible for rapid and enhanced secondary antibody responses upon a second exposure to an antigen [15]. It is speculated that the raised sIgA secretion rate for Run 2 compared with Run 1 reflects a secondary antibody response. Specifically, B cell sIgA memory, developed after Run 1, resulted in further enhanced antibody synthesis for Run 2 in response to dendritic cell antigen presentation.

Injury Immune Surveillance Mechanism

Finally, acute physical stress including muscle/tissue damage has been shown to induce blood leukocyte trafficking (migration) from the circulation to organs such as the skin, mucosal lining of the gastro-intestinal and urinary-genital tracts, lung, liver, and lymph nodes [36]. This migration has a protective nature and enhances "immune surveillance" in peripheral compartments [36]. It is tenable that a similar mechanism may underlie the increases in sIgA secretion rate observations in the present study. Specifically, as sIgA is the primary innate and adaptive immune component of the upper airways, an elevation in response to exercise-induced muscle/tissue damage would be a protective response to enhance immune surveillance and protection from infection in the upper airways. It is proposed that this is an evolutionarily conserved mechanism and is supported by recent research that demonstrated an innate, pre-programmed rapid sIgA increase after injury in both animals and humans, in gut-associated lymphoid tissue (GALT) and mucosa-associated lymphoid tissue (MALT) [24]. In subsequent studies, the same group demonstrated that injury and systemic inflammation stimulated airway sIgA increases via a mechanism that depended on the cytokines TNF-α, IL-1β, and IL-6 [27, 37].

Unfortunately, cytokines were not measured in the current study and, therefore, their hypothesised roles must remain conjecture; the lack of such data is acknowledged as a limitation of the study. Clearly, further investigation of this phenomenon and its relationship with exercise-induced muscle damage, neuroendocrine, cytokine, and cell migratory responses is warranted.

CONCLUSIONS

In summary, the present study is the first to show that a bout and two bouts of unaccustomed, eccentrically biased exercise induced alterations in salivary sIgA secretion rate that may serve as a protective adaptation to exercise-induced tissue damage. This finding suggests that the exercise-induced muscle damage from eccentrically biased endurance exercise may enhance mucosal immunity. This may occur via an evolutionarily conserved mechanism, regulated, in part, by cytokines and antigen-presenting cells, which serves to improve peripheral immune surveillance.

However, the detailed pathway(s) underlying this exercise-induced mechanism, as well as the secretory rate response to varying modes, duration and frequency of exercise, requires further investigation.

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