TITLE: The effect of fish oil, vitamin D and protein on URTI incidence in young active people

ABSTRACT

Upper respiratory tract infection (URTI) is a frequent illness among athletes. We investigated the effect of a multi-nutrient supplement (vitamin D, fish oil and protein) on the occurrence of URTI in young active people. Forty-two young recreational athletes were randomly assigned to receive either a supplementation (550 mg DHA, 550 mg EPA, 10 µg vitamin D3 and 8 g whey protein) or placebo for 16 weeks. Unstimulated saliva samples were collected by passive drool. Samples were analysed for IgA (sIgA) concentration and the secretion rate extrapolated by multiplying concentration by saliva flow rate. Physical activity levels and URTI incidence were monitored by questionnaire. Training status was not different between the 2 groups. There were no differences in the incidence, severity and duration of URTI. However the number of symptom days was lower in the supplemented compared to the control group (1.72 ± 1.67 vs 2.79 ± 1.76; $P < 0.05$). sIgA concentration and secretion rate did not differ between groups. This study demonstrates that 16 weeks of supplementation with fish oil, vitamin D and protein did not modify the incidence, severity and duration of URTI, although the total number of symptom days was reduced, in a healthy active population.

INTRODUCTION

One of the leading causes of a visit to a general practitioner is upper respiratory tract infections (URTI), such as coughs, colds, pharyngitis and laryngitis [11]. It has also been demonstrated that every adult will suffer, on average, between 2-5 colds every year [17] with a large economic cost in lost work days and medical expenses. Several factors can alter the incidence of URTI with periods of high intensity/duration exercise training being shown to result in an approximately 2 fold increase in URTI incidence [21,22]. This increase in URTI incidence has long been thought to be the result of perturbations of the immune system, with many changes to immune function after both acute and chronic exercise [28]. What has remained challenging, however, has been to establish a clear link between individuals with the greatest perturbations of the immune system and URTI incidence. In this regard one of the most promising makers associated with URTI incidence is the levels of salivary immunoglobin A (sIgA), with reduced sIgA having been demonstrated to be associated with URTI incidence in athletic groups [10]. It is of clear importance, therefore, to uncover strategies to reduce URTI incidence in athletic populations with nutrition often an area of focus.

In recent work vitamin D has been shown to be crucial in the regulation of the immune system [1] with lower levels of vitamin D being associated with an increase in respiratory infections and decrease in lung function in a middle aged British population [3]. Similar investigations have been carried out in athletic populations with athletes with low vitamin D status having a higher risk of URTI and suffering more symptoms when a URTI bout is present [15]. There are as yet no vitamin D supplementation studies to investigate whether such a strategy may reduce URTI incidence in such a population. In a similar vein it is known that fish oil can improve immune function in the recovery period after exercise [13], suggesting that fish oil supplementation may reduce URTI incidence although, as with vitamin D, there are no studies investigating this at present. One method which has successfully been shown to reduce URTI incidence is through an increase in dietary protein intake [29]. In this study, with a counterbalanced design, participants carried out 7 days of high intensity exercise training on either a normal (1.5g/kg body mass/day) or high protein diet (3.0g/kg body mass/day). The high protein diet attenuated the training induced rise in URTI incidence and restored leukocyte trafficking. Whilst this study shows clear potential this study was of short duration (7 days) with relatively small subject numbers (n=8) during a lab based induction of an overreached state and so more longer term studies in larger athletic populations during their normal training regimen are required.

The aim of the current study was to investigate the effects of supplementation with fish oil, vitamin D and protein on URTI incidence during a 16 week period in an athletic population and to determine whether such changes were associated with increases in sIgA.

METHODS

Participants

Forty two participants (Age 26.8 ± 4.4 years, height 171.6 ± 29.6 cm, body mass 68.2 ± 14.8 kg and percentage body fat 15.4 ± 6.9 %) were recruited to the study, with 16 females and 26 males. Two cohorts were recruited beginning in March 2013 (n=36) and September 2013 (n=6). All participants reported to be participating in at least 3 hours of moderate/high intensity exercise training per week. For the 16 week study period participants were asked to maintain their habitual exercise training and nutritional intake. The study was approved by the University of Aberdeen College of Life Sciences and Medicine Ethics Review Board and participants were made aware of the aims, risk and discomfort associated with the study before providing written informed consent [14]. Participants were free to withdraw at any time.

Supplementation

Participants were randomly assigned to either control (n=20; 9 females) or supplementation (n=22; 7 females) groups. Supplements consisted of drinks provided in unlabelled cartons with participants being blinded to which group they were assigned to. After their initial visit participants in the control group consumed 2 drinks per day, at a time of participants choosing, containing water, sugar, lingenberry juice (6.2%), red grape juice (3.9%), ascorbic acid, citric acid and lingonberry aroma. Each drink was 200 ml with 100 kcal of energy (25 g carbohydrate, 0 g fat and 0 g protein). The supplement group consumed 2 drinks per day with the same ingredients and each drink supplemented by 550 mg DHA, 550 mg EPA, 8 g whey protein isolate and 10 µg vitamin D3. Each drink was 200 ml with 200 kcal energy (28.5 g carbohydrate, 5.8 g fat and 8 g protein). Drinks were provided by Smartfish (Norway).

Experimental Protocol

Participants visited the laboratory, in the morning, at baseline and upon completion of the study. On both of these visits body mass was recorded to the nearest 0.1 kg using a weighing scale and height was measured to the nearest 0.5 cm using a stadiometer. Skinfold thickness was measured with callipers at four sites (bicep, tricep, subscapula and suprailiac) to the nearest 0.1 mm on the right side of the body. Body fat percentage was calculated using standard equations [7]. Unstimulated saliva samples were also collected, via 3 min of passive drool, at these visits.

Training load was monitored throughout the study period by asking participants to complete the standard short form of the International Physical Activity Questionnaire (IPAQ) every week. From this training load was calculated every week in metabolic equivalents (MET)min/week. Participants were also asked to complete a questionnaire to record URTI symptoms on a daily basis [8]. Participants were asked to take any medication, or visit their GP as normal when symptoms were present with this being recorded in their illness log. The illness log listed the following symptoms: sore throat, catarrh, runny nose, cough, fever, repetitive sneezing, joint aches and pains, general weakness, headache and loss of sleep. Severity ratings of mild, moderate and severe were given with the following guidance: Mild = normal training, moderate = modified/reduced training and severe = discontinued training); and these were scored as 1, 2 and 3, respectively, giving a quantitative total symptom score

for each day (calculated as symptoms multiplied by severity score). In any given week a symptom score of ≥12, and separated by at least one week from another week with a score ≥12, was taken as a single URTI episode. The total number of URTI symptom days was calculated as the number of days with a symptom score of ≥5 [23].

Saliva IgA

The volume of saliva collected in 3 minutes was estimated by weighing and the saliva flow rate subsequently calculated. Saliva was analysed for IgA using a commercially available ELISA kit (Salimetrics, USA) according to the manufacturer's instructions. Samples and standards were analysed in duplicate with the intra-assay coefficient of variation being 7.9%. Secretion rate for IgA was calculated by multiplying the saliva flow rate by the saliva IgA concentration.

Statistical Analysis

Differences in the number of participants who suffered an URTI episode between control and supplemented groups were assessed by a chi-square test. To compare the number of URTI episodes and total number of symptom days, and when a URTI was present to compare URTI duration and symptom severity score, independent t-tests were carried out. Changes in saliva IgA were compared using a two-way repeated measures ANOVA. Significance was accepted at $p<0.05$ and data are presented as mean ± SD.

RESULTS

Participants

Forty two participants were recruited to the study, with six participants fully withdrawing for a variety of reasons (e.g. injury, did not like the supplements, undisclosed reasons). A further six participants completed 12 weeks of the study, with thirty participants completing the full 16 weeks of the study, with saliva samples and questionnaires completed for all thirty six of these participants. Body mass increased slightly in the control group during the intervention (68.2 ± 14.5 kg at baseline to 69.2 ± 14.4 kg upon completion) with a slight decrease in the supplemented group (71.1 ± 9.8 kg at baseline to 69.2 ± 14.4 kg upon completion). Similarly percentage body fat increased slightly in the control group during the intervention (17.3 ± 5.0 % at baseline to 18.5 ± 5.0 % upon completion) with a slight decrease in the supplemented group (14.6 ± 7.8 % at baseline to 14.2 ± 7.1% upon completion). These small changes were not significant with the repeated measures ANOVA revealing no group, interaction or time effects in either body mass or percentage body fat (Table 1).

Training Loads

The weekly training load, estimated via the IPAQ, was not different between the two groups with an average weekly load of 3814 ± 2108 METmin/week in the control group and 3893 ± 2194 METmin/week in the supplemented group (Fig 1).

URTI Incidence and Severity

Data on URTI incidence and severity is presented in Fig 2. There were no differences in the number of URTI episodes between groups. There were no differences in the percentage of participants reporting URTIs during the study period, with 49% of participants in the control group and 45% of participants in the supplemented group reporting a URTI episode. When a URTI was present there were no differences in either the severity or the duration of the episode. There were also no differences in the number of visits to a GP (1 participant in control and 2 in the supplemented group) or number of times medication was taken (medication taken 7 times in the control group and 5 in the supplemented group). However, the total number of symptom days reports was significantly lower in the supplement group compared to the control group (1.72 ± 1.67 in the supplemented group vs 2.79 ± 1.76 in the control group; <0.05).

Salivary IgA

Figure 3 shows the sIgA concentration and secretion rate in control and supplemented groups upon entry to the study and upon completion of the intervention. There were no differences between either sIgA with either time or between the control and supplemented groups.

DISCUSSION

This 16 week study investigated the effects of a supplement containing fish oil, vitamin D and protein on self-reported URTI symptoms in a cohort of young active people. Throughout the trial participants engaged in their normal training and competition schedule and were instructed to maintain their normal dietary habits. The main finding of the current study was that the supplement had no effect on the incidence and duration of URTI episodes, or the severity of symptoms when an URTI was present. Furthermore sIgA concentration and secretion rate did not differ between groups. However, the supplemented group did report a lower number of symptom days when compared to the control group. Taken together this indicates that whilst this supplement has no effect on URTI the number of days with lower level symptoms, such as runny nose, cough, fever, catarrh, is reduced. As the guidance given to participants for scoring symptoms was related to the subsequent effect on training, this would still indicate that the supplement may be of use in the maintenance of training in this population. Clearly further work is required to test this directly.

These observed changes in symptom days, in the current study, were not associated with changes in sIgA, with no differences between groups either before or after the intervention. There is currently very little information on the effects of EPA, DHA, protein or vitamin D on sIgA levels and as the current study did not find any changes in URTI incidence, duration or severity perhaps the lack of change in sIgA is not surprising. It has previously been shown that sIgA secretion rate does decrease from “optimal” to “inadequate” vitamin D status [15], although to our knowledge there have been no previous supplementation studies. With regard protein supplementation one study has demonstrated that glutamine supplementation is unable to attenuate the acute exercise induced decrease in sIgA levels [18]. This however is the effect of a single amino acid on acute exercise responses and so the effect of chronic protein supplementation on sIgA levels remains to be tested. On the basis of the current study it looks unlikely that a very modest increase in protein would have any effect. Previous work has shown that, in mice, fish oil supplementation can preserve broncho-alveolar IgA levels in response to infusion with 5-fluorouracil, an anticancer drug known to reduce mucosal immunity [9]. However, in a similar vein to protein it would appear likely that in humans fish oil supplementation has very little effect on sIgA levels.

The supplement investigated in the current study was chosen on the basis of the immunomodulatory effects of the individual components. Whilst the current study is unable to determine which of these supplements is responsible for the reduction in total symptom days, it is prudent to discuss the literature available on the effects of the individual supplements on the immune system.

Fish oil, rich in EPA and DHA, has long been investigated for its effects on the immune system and its ability to reduce inflammation [4,16,25,27]. In exercise studies there are some conflicting results on the effects of fish oil consumption on such factors. Indeed in a study by Toft and colleagues [26] it was found that fish oil supplementation did not alter plasma IL-6 levels after a marathon, with similar results found after 3 days of intensive exercise [20]. However, we have recently demonstrated that fish oil supplementation can improve post-exercise peripheral blood mononuclear cell (PBMC) IL-2 production, NK cell activity and markers of oxidative stress [12,13]. No studies, thus far, have investigated the effects of EPA and/or DHA on URTI incidence in active populations.

Historically vitamin D was known for its role in the absorption of calcium in the intestine but in recent years the physiological roles of vitamin D have expanded manifold [2]. For example, Vitamin D has been demonstrated to promote antibacterial responses in monocytes/macrophages, altering T helper cell function and viral inactivation [24]. Consequently, the potential role of vitamin D in URTI incidence has been proposed due to the immune role of vitamin D. On this basis He and colleagues [15] investigated the incidence of URTI compared to plasma vitamin D levels. This study demonstrated that participants who were deficient in vitamin D (<30nmol/L 25(O)HD) had an increased number of symptom days and symptom severity score, with no significant effect on URTI incidence or duration. The findings do partially reflect the findings of the current study, indicating that supplementation with vitamin D may be contributing to the reduction in symptom days observed. However a major drawback of the current study was that we made no measure of plasma 25(O)HD and so have no information as to whether our cohort were vitamin D deficient at baseline and it would seem reasonable to assume that any beneficial effects of vitamin D supplementation would only be observed in a deficient group.

With regards the protein component of the supplement employed it is well established that amino acids are crucial substrate for lymphocytes and an important modulator of their function [5]. Indeed it has been shown that increasing dietary protein intake preserved neutrophil degranulation [6], superoxide production [19] and also that exercise induced impairments in leukocyte trafficking are restored [29]. In this latter study the incidence of URTI was also reduced after increasing dietary protein intake during a period of high intensity exercise training suggesting that dietary protein may be of importance in determining URTI incidence. It is worth pointing out that in the study of Witard and colleagues daily protein intake was increased by 118 g which is far in excess of 16 g of protein that was given in the current study. Whether this small increase in protein intake is a contributing factor, to the reduction in symptom days observed in the current study, remains to be established.

As mentioned above a major limitation of the current study was that no measure of baseline and post intervention plasma levels of EPA, DHA and vitamin D were made. We are therefore unable to determine whether levels of these were low at baseline or to monitor the increase post intervention.

In conclusion the current study has shown that a supplement containing fish oil, vitamin D and protein has no effect on the incidence or severity of URTI in a group of active people, although the total number of symptoms days was reduced. The current study was not designed to investigate the contributions of each of the components of this supplement and further work is needed in this area and also in determining the optimal quantities of each supplement.

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