Assessing Omega-3 Intake in Sport: the Brief Food Frequency Questionnaire and the Omega-3 Index in **Collegiate Women Soccer Players**

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Context: Omega-3 fatty acids modulate inflammatory processes and are considered beneficial for sport populations, highlighting a need to assess omega-3 intake in a practical manner. Food frequency questionnaires (FFQs) are inexpensive, noninvasive tools aimed at evaluating nutrient intakes such as omega-3 fatty acids. In healthy adults, a tailored, brief FFQ for estimating omega-3 intake was associated with the erythrocyte omega-3 fatty acid level, a biomarker for omega-3 tissue status and indicative of intake. However, the association between a brief omega-3 FFQ and erythrocyte levels, particularly the Omega-3 Index (eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], and EPA + DHA), has yet to be explored in a sport population.

Objective: To examine the association between omega-3 intake using a brief FFQ and the Omega-3 Index in collegiate women soccer players.

Design: Cross-sectional study. Setting: University sport team.

Patients or Other Participants: Thirty-one National Collegiate Athletic Association Division I collegiate women soccer players.

Main Outcome Measure(s): The brief omega-3 FFQ assessed dietary omega-3 intake: DHA and EPA. The Omega-Quant blood test measured erythrocyte omega-3 fatty acid (EPA, DHA) and Omega-3 Index (EPA + DHA) levels.

Results: Brief FFQ intakes of EPA, DHA, and EPA + DHA were positively correlated with the erythrocyte EPA (r=0.48, P=.007), DHA (r=0.73, P < .001), and Omega-3 Index (r=0.73, P < .001).

Conclusions: In a sample of collegiate women soccer players, the brief omega-3 FFQ was correlated with erythrocyte omega-3 fatty acid levels and may offer health practitioners a practical tool for assessing omega-3 intake in this collegiate sport population.

Key Words: athletes, nutrition, docosahexaenoic acid, eicosapentaenoic acid

Key Points

- Inadequate omega-3 dietary intake and suboptimal Omega-3 Index levels were observed in a sample of collegiate women soccer players.
- The brief omega-3 food frequency questionnaire was associated with erythrocyte omega-3 biomarkers and strongly associated with the Omega-3 Index.
- The instrument may be a practical tool for rapidly assessing omega-3 intake in a team sport setting.

icosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 polyunsaturated fatty acids important for modulating inflammatory processes within the body.¹ Eicosapentaenoic acid and DHA have been proposed to benefit athletic populations through their anti-inflammatory properties,² which include the ability to replace arachidonic acid in membrane phospholipids, alter inflammatory eicosanoid and cytokine release, and synthesize anti-inflammatory lipid mediators.¹ Increasing evidence²⁻⁴ in athletes also suggests that improving omega-3 status via supplementation can enhance muscle recovery; reduce exercise-related oxidative stress; and decrease levels of neurofilament light, a biomarker for axonal injury, after sport-related subconcussive head injuries. These physiological responses resulting from omega-3 polyunsaturated fatty acids' anti-inflammatory properties demonstrate a need for the practical assessment of omega-3 dietary intake

in athletic populations to better direct diet recommendations, supplementation recommendations, or both toward a more optimal omega-3 status during sport participation.

The Omega-3 Index is an erythrocyte biomarker, shown as a percentage of EPA + DHA in the total erythrocyte fatty acid content, commonly used to evaluate an individual's omega-3 status.⁵ The desirable range for the Omega-3 Index and omega-3 status with respect to cardioprotection is >8%.⁶ This biomarker has been used in a variety of populations, including athletes, to assess exposure to omega-3 intake.^{5,7,8} Unfortunately, routinely collecting Omega-3 Index levels can be time-consuming, invasive, and cost prohibitive; thus, alternative, more practical approaches to assessing omega-3 status are needed. Dietary intake, or a lack thereof, of EPA and DHA through food sources, supplementation, or both has been reported to be a primary factor that influences Omega-3 Index levels.^{5,7-10}

Nutrition

Therefore, evaluating dietary omega-3 intake is a feasible alternative measurement in determining status.

Food frequency questionnaires (FFQs) are an inexpensive, noninvasive tool for assessing an individual's omega-3 intake. In nonathletic populations, several variations of omega-3 FFQs have been validated against plasma and erythrocyte biomarkers^{10–14}; however, few authors^{9,15} have examined FFQs' utility in athletic populations. Answers to a 21-question FFQ by Sublette et al,¹³ specific for patients with major depressive disorder and healthy volunteers, demonstrated an association with plasma EPA and DHA as well as Omega-3 Index levels among female and male athletes participating in a wide range of sports.9,15 Currently, alternative omega-3 FFQs have yet to be considered and examined in sport populations. For example, a brief omega-3 FFQ, developed by DSM Nutritional Products, was validated for evaluating phospholipid and erythrocyte EPA and DHA levels in healthy adults.¹¹ This FFQ uses only 7 questions to calculate EPA and DHA intake from a variety of fish, seafood, egg, and poultry sources as well as supplementation. Thus, this specific brief FFQ may allow for a simple, more efficient assessment of omega-3 intake in team sport settings that often include many athletes.

The association between responses to this brief FFQ and erythrocyte omega-3 biomarkers of nutrient status, particularly the Omega-3 Index, among athletes has yet to be explored. Given a recent legislation change by the National Collegiate Athletic Association (NCAA), which reclassified omega-3 supplements as permissible for institutions to provide, this practical tool may also better inform health practitioners in identifying more targeted supplement dosage recommendations for various collegiate athletes and teams.¹⁶ Collegiate women soccer players may benefit from having their omega-3 intake and erythrocyte levels evaluated because of their high training loads, increased oxidative stress, and increased potential risk of sport-related head injuries.^{17,18} Therefore, the purpose of our study was to determine the relationship between estimated omega-3 intake from the brief FFQ and erythrocyte omega-3 fatty acid levels and Omega-3 Index in collegiate women soccer players. We hypothesized that (1) the FFQ-estimated EPA intake would be associated with erythrocyte EPA levels, (2) the FFQ-estimated DHA intake would be associated with erythrocyte DHA levels, and (3) the FFQ EPA + DHA intake would be associated with Omega-3 Index values.

METHODS

General Study Design

This was a cross-sectional study design with data collection before a competitive collegiate soccer season. Participants completed the brief omega-3 FFQ and provided a fingerstick blood sample for measurement of erythrocyte EPA, DHA, and the Omega-3 Index. All participants gave their informed consent, and the consent and study protocol were approved by the research team's university institutional review board.

Study Participants

Participants in this study were NCAA Division I collegiate women soccer players from 2 regional universi-

ties. A total of 31 players were enrolled. All current members of each soccer team were recruited to participate in the study. Players were excluded from the study if, within the month before data collection, they had begun omega-3 supplementation (EPA + DHA) of 1.2 g or more because the supplementation duration was not long enough for the erythrocyte omega-3 fatty acid membrane composition to reach a new steady state and reflect the increased omega-3 intake reported using the brief FFQ.¹⁹

Brief Omega-3 FFQ

The 7-question brief FFQ assessed omega-3 intake by grouping foods based on similar EPA and DHA contents.¹¹ Questions 1, 2, and 3 grouped fish and seafood sources based on high, medium, or low respective DHA and EPA content; 4, 5, and 6, liver, egg, and meat intake; and 7, dietary supplement use, including brand, dose, and frequency. For each question, participants estimated the total number of servings consumed over the previous month or week. Mean EPA and DHA intake for each question on the FFQ was determined using the US Department of Agriculture Nutrient Database.²⁰ We computed participants' daily EPA and DHA intake by multiplying the reported number of servings per question by the predetermined mean EPA and DHA content in each question.

Erythrocyte Omega-3 Fatty Acid Analyses

Dried blood spot supply kits and collection instructions for each participant were supplied by OmegaQuant Analytics, LLC, a Clinical Laboratory Improvement Amendments-certified laboratory that provides fatty acid and Omega-3 Index testing. A single drop of whole blood was collected from a fingerstick and placed on proprietary filter paper pretreated with antioxidant cocktail fatty-acid preservative solution and allowed to dry at room temperature for 15 minutes. Once collected, blood spot samples were mailed to OmegaQuant Analytics, LLC, for erythrocyte fatty acid analyses.

Statistical Analysis

Descriptive statistics for participants, omega-3 FFQ intake, and erythrocyte levels are summarized via frequencies, means, SDs, and ranges. Athletes were categorized based on the proposed Omega-3 Index risk zones for cardioprotection⁶: *high risk*, <4%; *intermediate risk*, 4% to 8%; or *low risk* (ie, desirable), >8%. Because the data did not satisfy the normality assumption, we used a nonparametric approach to analysis. Spearman rank correlation analyses were calculated to examine the relationships between the reported omega-3 intake from the brief FFQ (ie, DHA, EPA, and DHA + EPA), erythrocyte omega-3 fatty acids (EPA, DHA), and Omega-3 Index values. A *P* value of <.05 was considered statistically significant. We used SPSS (version 27; IBM Corp) to analyze the data.

RESULTS

Demographics are presented in Table 1. A summary of reported omega-3 intake from the brief FFQ and erythrocyte omega-3 fatty acid levels is given in Table 2. No participants described omega-3 supplementation >1 month

Table 1. Study Participants' Demographic Characteristics (N = 31)

Characteristic	Value
Height, mean ± SD, cm	169.8 ± 6.1
Student classification, No.	
Freshman	12
Sophomore	8
Junior	8
Senior	3
Playing position, No.	
Defender	11
Midfielder	13
Forward	4
Goalkeeper	3
Ethnicity, No.	
White	25
African American	4
Asian	1
Other	1

before data collection. Participants' mean dietary EPA intake was 95.85 \pm 82.58 mg/d and mean dietary DHA intake was $156.61 \pm 133.54 \text{ mg/d}$; thus, their total mean dietary omega-3 intake (EPA + DHA) was 252.46 ± 215.79 mg/d. The mean Omega-3 Index was 4.14% \pm 0.74%; a total of 48% (n = 15) had an Omega-3 Index of <4% or high risk, and 52% (n = 16) were categorized as having intermediate risk. No athletes had a desirable, or low-risk, Omega-3 Index >8%. Reported dietary intakes of EPA and DHA were positively correlated with estimated erythrocyte EPA (r = 0.48, P = .007) and DHA (r = 0.73, P< .001). Combined intake of EPA + DHA was positively correlated with the Omega-3 Index (r = 0.73, P < .001; Figure). The P values after multiple testing corrections using Bonferroni adjustments were .021, <.001, and <.001for EPA, DHA, and EPA + DHA, respectively.

DISCUSSION

To our knowledge, we are the first to examine the relationship between a brief omega-3 FFQ and erythrocyte omega-3 fatty acid levels, including the Omega-3 Index, in any sport population. The purpose of our study was to determine, in Division I women soccer players, if their estimated omega-3 intake using the respective brief FFQ was associated with omega-3 blood biomarkers, which indicate nutrient tissue status. In our sample, FFQ-reported dietary intakes of EPA, DHA, and EPA + DHA were significantly associated with the respective blood biomarkers: erythrocyte EPA, DHA, and Omega-3 Index. Furthermore, their mean estimated dietary omega-3 intakes were

Table 2. Erythrocyte and Dietary Omega-3 Characteristics

	Mean \pm SD	Range
Omega-3 fatty acids, %		
EPA	0.29 ± 0.13	(0.11–0.73)
DHA	2.18 ± 0.58	(1.41–3.39)
Omega-3 Index	4.14 ± 0.74	(3.17–6.01)
Brief omega-3 Food Frequency Questionnaire fatty acid intake, mg/d		
EPA	95.85 ± 82.58	(0-341.0)
DHA	156.61 ± 133.54	(0-569.0)
EPA + DHA	252.46 ± 215.79	(0–910.0)

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.



Figure. The relationship between athletes' erythrocyte omega-3 status and the Brief Omega-3 Food Frequency Questionnaire results for A, total eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid; B, total EPA; and C, total DHA. Spearman rank correlation analyses (2 sided) with line of best fit (P < .05).

inadequate (<300 mg/d) and Omega-3 Index values were suboptimal and essentially undesirable. Thus, the brief omega-3 FFQ may be an appropriate, practical, and necessary tool for evaluating omega-3 intake in athlete populations.

We found that the brief omega-3 FFQ answers correlated with the estimated erythrocyte EPA, DHA, and Omega-3 Index (Figure). The correlation coefficients of the abbreviated omega-3 FFQ with erythrocyte DHA and EPA were similar to those noted by Kuratko,¹¹ who was first to examine this brief FFQ in healthy adults. However, the relationship between the abbreviated omega-3 FFO and Omega-3 Index was not previously described. Conversely, Wilson and Madrigal¹⁵ used the 21-question omega-3 FFQ from Sublette et al¹³ to assess Omega-3 Index levels among a sample of collegiate athletes from 5 sport teams. They demonstrated an FFQ correlation coefficient with the Omega-3 Index $(r = 0.57, P < .01)^{15}$ that was comparable with the results we obtained using the brief FFQ. Furthermore, Ritz et al⁹ used the same 21-question FFQ by Sublette et al¹³ to evaluate the omega-3 status of collegiate athletes from multiple collegiate and regional sites. Omega-3 dietary intake via this FFQ also correlated with blood EPA (r = 0.34, P < .05), DHA (r = 0.40, P < .05) .05), and Omega-3 Index (r = 0.44, P < .05) levels in both female and male athletes. However, its correlation with Omega-3 Index levels was only moderate. Additionally, the Sublette et al¹³ FFQ, which is the only FFQ validated in sport populations, may have several limitations to generalizable sport application. The overall questionnaire design is specific for the New York region, which limits its use among other regions.¹³ The FFQ estimates omega-3 intake over the previous 6 months and lacks a more recent estimation of omega-3 dietary intake habits (ie, within the previous month). Also, it contains questions specific to α linolenic acid (ALA) sources, such as flax seeds, flaxseed oil, walnuts, and canola oil, which have not been associated with blood ALA levels.¹³ Given that ALA is not a component of the Omega-3 Index, ALA sources are not relevant when assessing omega-3 status because the conversion of ALA to EPA (5%-8%) and DHA (0.5%-5%) is relatively low, especially in individuals on a Western-style diet high in omega-6 fatty acids.^{9,13,15,21} In addition, the original FFQ was validated against plasma omega-3 levels and not specifically the erythrocyte biomarker (ie, Omega-3 Index).¹³ Lastly, considerable time is required for the reported dietary intake from the Sublette et al¹³ FFQ to be individually calculated based on portion size, frequency of consumption, sex, and average nutrient values of various omega-3 sources, which may limit its use by health care practitioners in sport team settings where many athletes routinely complete intake assessments. Thus, our findings suggest that this brief omega-3 FFQ may be an alternative or more preferred tool to use in sport populations versus the 21-question FFQ from Sublette et al.¹³ Furthermore, based on our results, this brief omega-3 FFQ could potentially be used as a proxy for athletes' Omega-3 Index levels. However, further research is warranted to confirm this predictive relationship.

In our sample, mean dietary EPA + DHA intake as assessed by the brief FFQ was 252 mg/d, which is low compared with the recommended intake of 500 mg/d set forth by the Academy of Nutrition and Dietetics.²² Similar

mean dietary EPA + DHA intakes have been observed in reports^{9,15} of FFQ-derived fatty acids among collegiate athletes. For example, Wilson and Madrigal¹⁵ identified a mean omega-3 intake of <100 mg/d, and Ritz et al⁹ described an intake of 141 mg/d among all analyzed collegiate athlete sport populations. Yet it is important to note that the Academy of Nutrition and Dietetics' suggested intake of EPA + DHA is not an established recommendation but simply a guideline that is in conjunction with other professional health organizations advice that omega-3 intake should range between 250 and 500 mg/d.²² Nevertheless, despite these omega-3 intake guidelines and recent NCAA legislation permitting omega-3 supplementation by institutions,¹⁶ low dietary consumption and subsequent suboptimal Omega-3 Index levels continue to be seen in collegiate sport populations, as evidenced by our sample of women soccer athletes. This further demonstrates the critical need to better monitor omega-3 intake and status to help educate sport athletes and institutions alike on the importance of including omega-3-rich foods and possibly supplementation of an athlete's or team's diet throughout sport participation.

Suboptimal and undesirable Omega-3 Index levels were present in this sample of collegiate women soccer athletes (Table 2). No Omega-3 Index values were within the desired range. These findings are similar to levels characterized in other sport populations.^{9,15,23,24} For example, in a cross-sectional analysis, researchers²⁴ assessed 106 German elite winter endurance athletes' Omega-3 Index levels. Only 1 athlete had an Omega-3 Index level within the desired range, whereas the remaining athletes fell below the target range (4.97% \pm 1.19%). In a large cohort of collegiate American football athletes (N = 404), Omega-3 Index levels were also below the desired range (4.4% \pm 0.8%), with 34% (n = 138) of the sample categorized as high risk (ie, <4%), 66% (n = 266) as intermediate risk (ie, 4% to 8%), and none as low risk or having a desirable level (ie, >8%).²³ Moreover, a large cross-sectional analysis⁹ of collegiate athletes (N = 1528) participating in a wide range of sports revealed Omega-3 Index levels of $4.3\% \pm 0.81\%$, with 38% categorized as high risk, 68% as intermediate risk, and no athletes within the low-risk or desired Omega-3 Index range. Among the soccer athletes in our current study, 48% were categorized as high risk, 52% as intermediate risk, and none as low risk. Our findings are in accordance with those in athlete populations sampled earlier and suggest that undesirable omega-3 index levels exist across all collegiate athletic populations, regardless of sex and sport. Thus, our results further highlight the need among collegiate athletic populations for a quick nutrientassessment tool to evaluate dietary omega-3 intake in order to better direct dietary recommendations, supplementation recommendations, or both and optimize athletes' omega-3 status to enhance not only cardioprotection but also antiinflammatory properties during sport participation that may positively influence sport performance and overall health.

Strengths and Limitations

Strengths of our work include the use of the OmegaQuant blood spot test kit, which has been used in other omega-3 FFQ validation studies,^{9,15,25} to assess estimated erythrocyte EPA, DHA, and Omega-3 Index. Additionally, erythrocyte

biomarkers were measured instead of plasma fatty acid levels, which are more reflective of an individual's long-term omega-3 status.^{19,26} Also, the brief omega-3 FFQ is simple, efficient, and easy for participants to complete in a collegiate team sport setting. Lastly, ALA intake was not assessed in the brief FFQ, which may allow for more accurate assessment of omega-3 status because ALA intake has not been correlated with plasma or erythrocyte fatty acids.9-15,25 Despite these strengths, several limitations should be noted. The study sample was small (N = 31) and consisted of only collegiate women soccer athletes. Therefore, future investigations are needed to assess other sport populations as well as male athletes, particularly those who are provided more frequent omega-3 supplementation by their institutions, to enhance the generalizability of these initial findings. Additionally, though we reported a statistically significant relationship between the brief FFO and Omega-3 Index, which aligned with previous findings, several factors aside from dietary intake have been shown to influence an individual's Omega-3 Index, including sex, age, weight, smoking, aspirin use, low-density lipoprotein cholesterol, physical activity, and higher education levels^{10,27,28}; given our study aims, we did not control these factors. Future authors should account for these factors that may influence Omega-3 Index values in addition to dietary intake to fully elucidate the potential accuracy of using FFQ-derived omega-3 intake to indicate an athlete's omega-3 status and potential predictive relationship with Omega-3 Index values.

CONCLUSIONS

Overall, estimated omega-3 dietary intakes in collegiate women soccer players using the brief omega-3 FFQ were related to omega-3 biomarkers of status. These findings suggest that this brief omega-3 FFQ may be an appropriate tool for health practitioners to use when evaluating omega-3 intake, and likely omega-3 status, in this collegiate sport population so they can make proper recommendations regarding diet and supplementation.

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