Prostaglandins, Leukotrienes and Essential Fatty Acids 90 (2014) 199–206

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

# Temporal changes in human skeletal muscle and blood lipid composition with fish oil supplementation

Chris McGlory [b,a], Stuart D.R. Galloway [a], D. Lee Hamilton [a], Craig McClintock [a], Leigh Breen [b,c], James R. Dick [d], John G. Bell [d], Kevin D. Tipton [a,*]

[a] Health and Exercise Sciences Research Group, University of Stirling, Scotland, UK
[b] Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Ontario, Canada
[c] School of Sport and Exercise Sciences, University of Birmingham, UK
[d] Institute of Aquaculture, University of Stirling, Scotland, UK

ARTICLE INFO

*Article history:*
Received 10 January 2014
Received in revised form
17 February 2014
Accepted 19 March 2014

*Keywords:*
n-3 PUFA
Muscle
FAK

ABSTRACT

The aim of this study was to examine changes in the lipid profile of red blood cells and muscle tissue along with the expression of anabolic signalling proteins in human skeletal muscle. Following a 2-week control period, 10 healthy male participants consumed 5 g $d^{-1}$ of fish oil (FO) for 4 weeks. Muscle biopsies and venous blood samples were collected in the fasted state 2 weeks prior (W-2) and immediately before (W0) the initiation of FO supplementation for internal control. Muscle biopsies and venous blood samples were again obtained at week 1 (W1), 2 (W2) and 4 (W4) during FO supplementation for assessment of changes in lipid composition and expression of anabolic signalling proteins. There was no change in the composition of any lipid class between W-2 and W0 confirming control. Following FO supplementation n-3 polyunsaturated fatty acid (n-3 PUFA) muscle lipid composition was increased from W0 to W2 and continued to rise at W4. n-3 PUFA blood lipid composition was increased from W0 to W1 and remained elevated for the remaining time points. Total protein content of focal adhesion kinase (FAK) increased from W0 to W4 whereas total mechanistic target of rapamycin (mTOR) was increased from W0 at W1 with no further significant increases at W2 and W4. These data show that FO supplementation results in discordant changes in the n-3 PUFA composition of skeletal muscle compared to blood that is associated with increases in total FAK content.


## 1. Introduction

The consumption of food rich in Omega n-3 polyunsaturated fatty acids (n-3 PUFA) is thought to be beneficial for many cardiovascular disease risk factors, including blood pressure and [1] immune function [2]. There are also other clinically relevant health claims associated with the consumption of n-3 PUFA [3–5] that are concomitant with increases in the n-3 PUFA composition of the associated biological tissues [3,6–10]. However, whilst time course increases in the n-3 PUFA composition of erythrocytes [11], platelets, buccal cells, mononuclear cells and adipose tissue with n-3 PUFA supplementation have recently been established [10], to date, no study has characterised such a time course in human skeletal muscle.

Whilst much is known about other tissues, far less is known about the impact of n-3 PUFA supplementation on human skeletal muscle. Some data exist to show that n-3 PUFA supplementation may alter respiration kinetics and render skeletal muscle more sensitive to the effects of insulin [7,12]. Moreover, work in rodents has demonstrated that dietary fish oil alleviates *soleus* muscle atrophy during a period of enforced immobilization [13]. In humans, supplementation with n-3 PUFA-rich fish oil for 8 weeks is reported to enhance muscle protein synthesis rates (MPS) in response to a hyperaminoacidemic–hyperinsulinemic infusion in both the young and elderly [8,9]. Moreover, there is evidence that n-3 PUFA supplementation augments strength gains in response to resistance training in elderly humans [14]. Mechanistically, the anabolic influence of n-3 PUFA supplementation is purported to be mediated by enhanced mechanistic target of rapamycin (mTOR)-p70 ribosomal protein S6 kinase (p70S6K) signalling [8,9]. Indeed, changes in mTOR-p70S6K signalling with fish oil supplementation in those studies were also shown to be accompanied by significant increases in the n-3 PUFA composition of skeletal muscle [8,9]. Thus, it appears that n-3 PUFA supplementation increases the n-3 PUFA composition of skeletal muscle, which may confer an anabolic influence in part, via mTOR-p70S6K signalling. However, whether fish oil supplementation alters the expression of these proteins and or other mechanically sensitive proteins remains uncertain.

* Corresponding author.
*E-mail addresses:* Christopher.mcglory@stir.ac.uk, Chris.mcglory@btinternet.com (C. McGlory), k.d.tipton@stir.ac.uk (K.D. Tipton).

http://dx.doi.org/10.1016/j.plefa.2014.03.001
0952-3278/

Although there are now a growing number of studies that characterise changes in the n-3 PUFA composition of skeletal muscle with n-3 PUFA supplementation [8,9,12,15,16], many of these studies are limited to pre- and post-supplementation measurements with little temporal resolution. Data are available on the time course of n-3 PUFA changes in blood [11] and adipose tissue with [10] n-3 PUFA supplementation; however, to our knowledge, no study has established the time course of n-3 PUFA changes in human skeletal muscle. Given the potential beneficial impact of increasing the skeletal muscle n-3 PUFA composition on metabolic health [3,7], data that demonstrate a time course increase in skeletal muscle n-3 PUFA composition with fish oil supplementation could therefore provide critical data for clinical and athletic practice. Thus, the primary aim of the present investigation was to identify the time course of n-3 PUFA change in skeletal muscle over 4 weeks of n-3 PUFA-enriched fish oil supplementation. In addition, as previous reports demonstrate improved strength gains during resistance training [14] and changes in mTOR-p70S6K signalling with n-3 PUFA supplementation [8,9,13,17], a secondary aim was to determine whether 4 weeks of fish oil supplementation modified the expression and or phosphorylation of key anabolic intramuscular signalling proteins (FAK, mTOR, p70S6K, and 4E-BP1).

## 2. Materials and methods

### 2.1. Participants

Ten healthy, moderately active males who participated in team sports recreationally (aged 21 ± 3 yrs; body mass 76 ± 4 kg, mean ± SEM) from the University of Stirling and the surrounding area volunteered to participate in the present investigation. Following health screening, participants were excluded if they were engaged in any form of dietary supplementation or were taking any prescribed medication. This study was conducted according to the guidelines laid down in the Declaration of Helsinki (2008) and the Local Ethics Committee, University of Stirling, approved all procedures. Written, informed consent was obtained prior to the commencement of the experiment.

### 2.2. Experimental design

In a one-way, repeated measures design, participants reported to the laboratory on five separate occasions. Initial baseline assessment of muscle and blood lipid profiles was conducted at −2 (W-2) and 0 (W0) week, to determine muscle and blood lipid profiles over a period of habitual diet and physical activity; thus, participants served as their own internal control (Tables 1 and 2). By employing a one-way, repeated measures design, we were able to circumvent issues such as genetic variability between participants and statistical power associated with a between-groups approach. Following this baseline control period, participants consumed 5 g $d^{-1}$ of fish oil capsules (providing 3500 mg EPA [20:5n-3]; 900 mg DHA [22:6n-3] and vitamin E 0.1 mg, Ideal Omega-3, Glasgow Health Solutions Ltd, UK; see Supplementary Table 1 for full fatty acid profile) for 4 weeks. The supplemental fish oil dose and participant number were chosen based on previous work showing that a similar dose in 10 males can induce significant changes in the lipid profile of human blood over a

**Table 1**
Full muscle lipid profiles.

| Fatty acid | −2 week | 0 week | 1 week | 2 week | 4 week |
|---|---|---|---|---|---|
| 14:0 | 0.95 ± 0.04 | 0.93 ± 0.05 | 0.95 ± 0.05 | 0.99 ± 0.08 | 0.85 ± 0.03 |
| 15:0 | 0.23 ± 0.01 | 0.21 ± 0.01 | 0.26 ± 0.01 | 0.20 ± 0.01 | 0.21 ± 0.01 |
| 16:0 | 17.49 ± 0.27 | 17.59 ± 0.31 | 18.03 ± 0.26 | 17.55 ± 0.37 | 17.71 ± 0.36 |
| 18:0 | 11.41 ± 0.27 | 11.19 ± 0.26 | 11.16 ± 0.17 | 10.98 ± 0.35 | 11.66 ± 0.16 |
| 20:0 | 0.13 ± 0.01 | 0.13 ± 0.01 | 0.13 ± 0.01 | 0.13 ± 0.01 | 0.13 ± 0.01 |
| 22:0 | 0.21 ± 0.02 | 0.17 ± 0.01 | 0.17 ± 0.02 | 0.16 ± 0.02 | 0.19 ± 0.02 |
| 24:0 | 0.18 ± 0.01 | 0.20 ± 0.02 | 0.15 ± 0.01 | 0.15 ± 0.02 | 0.17 ± 0.02 |
| **Total saturated** | **30.59 ± 0.26** | **30.42 ± 0.31** | **30.86 ± 0.21** | **30.15 ± 0.21** | **30.92 ± 0.34** |
| 16:1n-9 | 0.40 ± 0.02 | 0.39 ± 0.02 | 0.34 ± 0.01 | 0.42 ± 0.02 | 0.35 ± 0.01 |
| 16:1n-7 | 1.22 ± 0.14 | 1.40 ± 0.13 | 1.24 ± 0.11 | 1.49 ± 0.17 | 1.03 ± 0.10 |
| 18:1n-9 | 15.93 ± 1.06 | 16.58 ± 0.99 | 17.25 ± 0.90 | 17.69 ± 1.39 | 14.38 ± 0.76 |
| 18:1n-7 | 1.86 ± 0.03 | 1.91 ± 0.02 | 1.88 ± 0.03 | 1.89 ± 0.05 | 1.80 ± 0.03 |
| 20:1n-9 | 0.24 ± 0.02 | 0.24 ± 0.02 | 0.25 ± 0.02 | 0.25 ± 0.03 | 0.21 ± 0.02 |
| 24:1n-9 | 0.22 ± 0.01 | 0.25 ± 0.02 | 0.21 ± 0.02 | 0.23 ± 0.06 | 0.19 ± 0.01 |
| **Total monounsaturated** | **19.87 ± 1.15** | **20.76 ± 1.08** | **21.16 ± 0.96** | **21.97 ± 1.53** | **17.96 ± 0.81** |
| 18:2n-6 | 24.27 ± 0.47 | 23.75 ± 0.26 | 23.55 ± 0.56 | 22.91 ± 0.59 | 23.18 ± 0.40 |
| 18:3n-6 | 0.08 ± 0.00 | 0.08 ± 0.00 | 0.07 ± 0.00 | 0.07 ± 0.00 | 0.07 ± 0.00 |
| 20:2n-6 | 0.27 ± 0.01 | 0.27 ± 0.01 | 0.26 ± 0.01 | 0.24 ± 0.01 | 0.24 ± 0.01 |
| 20:3n-6 | 1.14 ± 0.06 | 1.14 ± 0.07 | 1.07 ± 0.05 | 1.04 ± 0.07 | 1.04 ± 0.06 |
| 20:4n-6 | 10.47 ± 0.50 | 10.46 ± 0.68 | 9.75 ± 0.54 | 9.69 ± 0.69 | 9.98 ± 0.63 |
| 22:4n-6 | 0.54 ± 0.04 | 0.53 ± 0.04 | 0.52 ± 0.05 | 0.50 ± 0.03 | 0.46 ± 0.04 |
| 22:5n-6 | 0.23 ± 0.02 | 0.22 ± 0.02 | 0.24 ± 0.01 | 0.20 ± 0.02 | 0.18 ± 0.02 |
| **Total n-6 PUFA** | **36.99 ± 0.83** | **36.45 ± 0.82** | **35.46 ± 0.71** | **34.64 ± 1.13** | **35.12 ± 0.56** |
| 18:3n-3 | 0.49 ± 0.03 | 0.51 ± 0.05 | 0.54 ± 0.02 | 0.58 ± 0.04 | 0.47 ± 0.03 |
| 20:5n-3 | 0.61 ± 0.05 | 0.59 ± 0.05 | 0.94 ± 0.08 | 1.36 ± 0.11 | 2.35 ± 0.22 |
| 22:5n-3 | 1.28 ± 0.04 | 1.24 ± 0.05 | 1.29 ± 0.04 | 1.46 ± 0.08 | 1.77 ± 0.09 |
| 22:6n-3 | 1.49 ± 0.17 | 1.47 ± 0.16 | 1.49 ± 0.16 | 1.69 ± 0.14 | 2.13 ± 0.21 |
| **Total n-3 PUFA** | **3.86 ± 0.23[a]** | **3.80 ± 0.22[a]** | **4.28 ± 0.24[a,b]** | **5.14 ± 0.28[b]** | **6.79 ± 0.46[c]** |
| 16:0DMA | 5.37 ± 0.22 | 5.29 ± 0.21 | 5.10 ± 0.22 | 5.18 ± 0.29 | 5.68 ± 0.20 |
| 18:0DMA | 1.77 ± 0.08 | 1.78 ± 0.07 | 1.68 ± 0.07 | 1.72 ± 0.08 | 1.90 ± 0.07 |
| 18:1DMA | 1.55 ± 0.07 | 1.50 ± 0.06 | 1.46 ± 0.05 | 1.32 ± 0.13 | 1.63 ± 0.07 |
| **Total DMA** | **8.69 ± 0.34** | **8.57 ± 0.29** | **8.24 ± 0.31** | **8.22 ± 0.42** | **9.21 ± 0.29** |

Total saturated fatty acids, total monounsaturated fatty acids, total n-6 polyunsaturated fatty acids (PUFA), total n-3 PUFA and total dimethyl aldehyde (DMA). Values are % total fatty acids mean ± SEM. Means that do not share a letter are significantly different from one another ($p < 0.05$).