Elevating the omega-3 index in older adults: a nutritional intervention to optimise cardiovascular and physical function during aging

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Elevating the omega-3 index in older adults: a nutritional intervention to optimise cardiovascular and physical function during aging

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Master of Philosophy

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The University of Wollongong
School of Medicine

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ABSTRACT

Background: As adults age, there is a progressive decline in cardiovascular and skeletal muscle function that directly impacts on physical capacity and exercise tolerance. In the elderly, an increase in energy cost of walking is associated with slower walking speeds and augmented fatigue. Both cardiovascular function, and in particular heart rate, and physical function such as walking speed have strong predictability on longevity in this cohort.

Evidence supports that the long chain omega-3 polyunsaturated fatty acid (LC n-3 PUFA), docosahexaenoic acid (DHA), incorporates into cardiac and skeletal muscle at a dose that is achievable via fatty fish consumption. In the case of skeletal muscle there is certainly evidence supporting the improvement of muscular strength at high doses. In fact, population health is improved by the regular consumption of fatty fish and this is reinforced by direct measures of physical capacity. In the laboratory, the effect of elevating the omega-3 status of an individual includes improved heart and skeletal muscle function especially when physiological strain is increased and supports the need for increased intake of LC n-3 PUFA, especially DHA which is found in abundance in contractile cells.

Objective: This study sought to determine whether low dose (2g/day), DHA-rich fish oil could elevate the red blood cell (RBC) omega-3 status and as a result modify heart function and improve physical capacity in physically active and healthy older adults.

Design: Using randomised placebo-controlled trial, physically fit older adults aged 60-85 years were supplemented with either 2g/d of DHA-rich fish oil (FO) (delivering 560mg/d DHA and 140mg/d eicosapentaenoic acid (EPA)), or 2g/d of placebo oil (control) (Sunola oil) for 16 weeks. Omega-3 index (RBC EPA+DHA) was measured before and after supplementation. Participants
completed a series of physical function tests, including walking speed over distances of 20-400m. In addition, a submaximal treadmill ramp protocol was conducted to evaluate the heart rate and oxygen cost of walking. Most importantly, heart rate was measured during both rest (including overnight sleep), exercise states and recovery.

**Results:** In physically fit and healthy older adults (mean walking speed >1.5m/s), the omega-3 index (%) was significantly increased (P<0.01) after fish oil supplementation (n=9) and not placebo (n=8) (control: pre 6.06±0.29, post 5.88±0.18; FO: pre 6.01±0.23, post 8.31±0.37 %). As a result, fish oil supplementation significantly lowered heart rate during steady state over ground walking in the 400m walk test (control: pre 120±2, post 124±2; FO: pre 122±2, post 115±2 bpm, P<0.01, height as a covariate) with no modification to self-selected walking speed. In addition, fish oil supplementation also lowered overnight sleeping heart rate (control: -0.2±1.0; fish oil: -3.8±1.2 bpm, P<0.05) without change to the heart rate variability (P>0.05). There was no effect of increasing the omega-3 index on the oxygen cost of walking (P>0.05) which might help to explain the non-modification to walking speed (P>0.05) in this already very active group. Additionally, physical function tasks, including grip strength and timed up and go were not substantially modified as a result of fish oil supplementation (P>0.05) and may too be due the high physical function in this group.

**Conclusions:** This study established that a DHA-rich fish oil dose that is reproducible through consumption of two fish meals per week significantly improves the omega-3 index (RBC EPA+DHA) in older adults. This increase in the omega-3 index resulted in improved cardiovascular function as reflected by the lowering of heart rate, particularity during fast walking. This study has supported the need to further promote LC n-3 PUFA in the diets of older adults and highlights this can be achieved via the consumption of regular fish that is rich in DHA.
Acknowledgements

Thank you to all those who were involved with my study. Especially my supervisors, Dr Gregory Peoples, Prof Peter McLennan, A/Prof Karen Walton, and Mr Marc Brown for educating and guiding me throughout this thesis. Thank you to the research students who assisted with data collection, and to the participants who took part in the study.
Certification

I, Ryan Anthony, declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Master of Philosophy, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Ryan Anthony
28th August 2018
# TABLE OF CONTENTS

ABSTRACT ................................................................................................................................. i

TABLE OF CONTENTS ............................................................................................................... v

LIST OF FIGURES ...................................................................................................................... viii

LIST OF TABLES ........................................................................................................................ ix

1. Introduction ........................................................................................................................... 1
   1.1 Conceptual overview ........................................................................................................ 1
   1.2 Functional decline with age .............................................................................................. 4
       1.2.1 Sarcopenia and declining strength ............................................................................. 4
       1.2.2 Cardiovascular decrements during aging ................................................................. 5
       1.2.3 Oxygen cost of exercise and the impact of aging .................................................... 7
   1.3 The influence of diet and nutrition behaviours on health and longevity ....................... 8
   1.4 Fish oil providing long-chain omega-3 polyunsaturated fatty acids ............................ 9
       1.4.1 Fish consumption: Current intakes and trends ......................................................... 10
       1.4.2 Fish oil and cardioprotection .................................................................................... 11
       1.4.3 Fish oil and sarcopenia ............................................................................................ 12
       1.4.4 Fish oil and oxygen efficiency .................................................................................. 17
   1.5 Aims and hypothesis ........................................................................................................ 18

2. General Methods .................................................................................................................. 20
   2.1 Study design ..................................................................................................................... 20
   2.2 Experimental overview .................................................................................................... 20
   2.3 Participant recruitment and selection ............................................................................. 22
   2.4 Participant allocation to groups ....................................................................................... 25
   2.5 Dietary supplementation ................................................................................................. 25

3. Laboratory assessments ........................................................................................................ 27
   3.1 Overview .......................................................................................................................... 27
   3.2 Anthropometry and participant preparation .................................................................... 27
   3.3 Assessment of nutritional status and dietary intake ....................................................... 28
   3.4 Senior fitness assessments ............................................................................................... 29
       3.4.1 30 second arm curl ..................................................................................................... 29
       3.4.2 Grip strength .............................................................................................................. 29
       3.4.3 30 second Chair stand ............................................................................................... 30
       3.4.4 Time up and go ......................................................................................................... 30
   3.5 Walking assessments ......................................................................................................... 30
3.5.1 Experimental measures and analysis .......................................................... 31
3.5.1.1. Speed .................................................................................................. 31
3.5.1.2. Heart rate .......................................................................................... 31
3.5.1.3. Muscle Oxygen .................................................................................. 32
3.6 Submaximal treadmill test ........................................................................... 33
3.6.1 Pre-exercise ............................................................................................. 33
3.6.2 Treadmill protocol ................................................................................... 33
3.6.3 Post treadmill recovery phase .................................................................. 34
3.6.4 Experimental measures and analysis ..................................................... 34
3.6.4.1. Heart rate .......................................................................................... 34
3.6.4.2. Heart rate recovery ............................................................................ 34
3.6.5 Muscle oxygen ......................................................................................... 37
3.6.6 Oxygen consumption ............................................................................... 37
3.6.7 Rating of perceived exertion .................................................................. 38
3.7 Blood sample collection and storage .......................................................... 38
4. Ambulatory cardiac monitoring .................................................................... 41
4.1 Overview ..................................................................................................... 41
4.2 Protocol ....................................................................................................... 41
4.3 Analysis ....................................................................................................... 41
4.3.1 Time domain heart rate variability ........................................................ 42
4.3.2 Nonlinear domain heart rate variability ............................................... 42
4.3.3 Frequency domain heart rate variability ............................................... 43
4.4 Experimental standardisation ..................................................................... 45
4.5 Statistical analysis ....................................................................................... 45
5. Results ........................................................................................................... 46
5.1 Demographics and functional capacity ....................................................... 46
5.2 The oxygen cost of walking in the older adult ........................................... 49
5.3 Agreement between self-reported nutrition data and blood fatty acids ...... 54
5.4 Agreement between physical function and blood fatty acids ................... 56
5.5 Fish oil supplementation and the effects on physiological and physical function ...... 57
5.6 Diet assessment .......................................................................................... 58
5.7 Fatty acids .................................................................................................. 60
5.8 Senior Fitness Assessment ........................................................................... 64
5.9 Submaximal treadmill protocol ................................................................... 69
5.10 Ambulatory 24-hour heart rate monitoring .......................................................... 73
6. Discussion .................................................................................................................. 77
   6.1 Aged physiology .................................................................................................... 77
   6.2 Long-chain omega-3 polyunsaturated fatty acids: intake and supplementation ........ 79
      6.2.1 Determination of omega-3 status ................................................................. 79
      6.2.2 Dose and composition of supplemental omega-3 fatty acids ......................... 81
   6.3 Effect of fish oil on cardiovascular outcomes at rest and exercise ....................... 83
   6.4 Effect of fish oil on physical function and strength measures ......................... 88
   6.5 Effect of fish oil on oxygen cost of exercise ....................................................... 90
   6.6 Summary ............................................................................................................ 93
   6.7 Limitations .......................................................................................................... 93
   6.8 Recommendations for future studies ................................................................. 94
   6.9 Conclusion ......................................................................................................... 95
7. References .............................................................................................................. 96
LIST OF FIGURES

Figure 2.1. Experimental overview................................................................. 21
Figure 2.2. Participant screening, recruitment and completion flow chart...................... 24
Figure 3.1. Net heart rate (mean ± SEM) during post-exercise recovery.......................... 36
Figure 3.2. Heart rate log values (mean ± SEM) during post-exercise recovery.................. 36
Figure 3.3. Logit heart rate values (mean ± SEM) during post-exercise recovery............... 36
Figure 4.1. Example of a Poincaré plot generated from baseline data.......................... 44
Figure 5.1. Linear regression of 20m usual walking speed and oxygen cost of walking at (A) stage one (A) and stage two (B) of treadmill walking, 20m rapid walking speed and oxygen cost of walking at stage one (C) and stage two (D) of treadmill walking, and 400m rapid walking speed and oxygen cost of walking at stage one (E) and stage two (F) of treadmill walking. Treadmill stage one (n=19), treadmill stage two (n=15). .................................................................................. 51
Figure 5.2. Linear regression of heart rate and oxygen cost of walking at stage one (A) and stage two (B) of the treadmill protocol .......................................................... 52
Figure 5.3. Whole blood EPA, DHA, and red blood cell equivalent omega-3 index for control (n=8) and fish oil (n=9) groups, pre and post supplementation ........................................... 62
Figure 5.4. Walking speed, heart rate, and tissue saturation index during the 400m walk test .. 67
Figure 5.5. Heart rate collected before and after supplementation during the 400m walk test in the control (n=8) and fish oil (n=9) group. .......................................................... 68
Figure 5.6. Heart rate change collected via a cardiac monitor during overnight sleep in control (n=8) and fish oil (n=9) groups. $P=0.28$. .......................................................... 73
Figure 5.7. Heart rate change during the lowest ten minute heart rate period overnight......... 75
LIST OF TABLES

Table 2.1. Major fatty acid percentage composition of the dietary supplements used in the study ................................................................. 26
Table 5.1. Anthropometric and physiology data at baseline for the entire cohort ................................................................. 47
Table 5.2. International Physical Activity Questionnaire data for males (n=9) and females (n=13) at baseline for the entire cohort (n=22) ................................................................. 48
Table 5.3. Association between walking speed and age, height and BMI for the entire cohort (n=22) ................................................................. 50
Table 5.4. Association between walking speed and treadmill oxygen cost of walking ................................................................. 50
Table 5.5. Linear regression of heart rate and oxygen cost of walking during the treadmill protocol ................................................................. 52
Table 5.6. Linear regression of treadmill oxygen cost of walking and self-reported physical activity and fatigue ................................................................. 53
Table 5.7. Relationship between whole blood fatty acids and red blood cell equivalent omega-3 index and self-reported fatty acids from the PUFA food frequency questionnaire ................................................................. 54
Table 5.8. Relationship between whole blood fatty acids and red blood cell equivalent omega-3 index and self-reported fatty acids from the 3-day food record ................................................................. 55
Table 5.9. Relationship between self-reported fatty acids from the 3-day food record and PUFA food frequency questionnaire ................................................................. 55
Table 5.10. Relationship between whole blood fatty acids and red blood cell equivalent omega-3 index at baseline and heart rate, oxygen cost of treadmill walking, walking speed, and grip strength ................................................................. 56
Table 5.11. Anthropometric and demographic data, before and after supplementation ................................................................. 57
Table 5.12. Nutrition data collected from the 3-day food record for control (n=7) and fish oil (n=7) groups ................................................................. 59
Table 5.13. Fatty acid composition expressed as a percent of total identified fatty acids from a whole blood, dry sample ................................................................. 61
Table 5.14. Bodyweight-adjusted capsule dose compared to change in long-chain fatty acids in whole blood and omega-3 index in red blood cells ................................................................. 63
Table 5.15. Physiological and senior fitness assessment data, before and after supplementation ................................................................. 65
Table 5.16. Omega-3 index and overnight heart rate in those that did (n=5) and did not meet (n=12) normative walking speed for age and gender ................................................................. 65
Table 5.17. Self-reported physical activity (IPAQ) and physical and mental fatigue (PFS) ................................................................. 66
Table 5.18. Heart rate recovery (heart beat drop) at 1 minute and 2 minutes following the 400m walk test ................................................................. 69
Table 5.19. Heart rate, oxygen consumption, oxygen cost of walking, tissue saturation index and rating of perceived exertion data collected during the submaximal treadmill protocol ................................................................. 70
Table 5.20. Carbohydrate oxidation rate, fat oxidation rate and respiratory exchange ratio at rest and during stage one and stage two of treadmill walking ................................................................. 71
Table 5.21. Heart rate recovery following the treadmill walking protocol ................................................................. 72
Table 5.22. Overnight heart rate and heart rate variability parameters ................................................................. 74
Table 5.23. Heart rate and heart rate variability parameters collected during the lowest ten minute heart rate period overnight ................................................................. 76
1. Introduction

1.1 Conceptual overview

The course of normal aging results in an alterable reduction in functional capacity of multiple physiological systems, including cardiovascular and skeletal muscle (Trombetti, Reid et al. 2016, Jakovljevic 2018). There is a range of physiological factors that influence functional status of the elderly. These include cardiorespiratory declines that result in a coordinated de-adaptation of oxygen delivery and utilisation (Daley and Spinks 2000, Burtscher 2013). Throughout adult life, beginning in approximately the fourth decade (Janssen, Heymsfield et al. 2000), there is a decline in aerobic capacity and an increase in energy cost of walking which leads to a slower walking speed which serves to reduce energetic requirements and attenuate fatigue (Wert, Brach et al. 2013). Preferred walking speed slows with age and is a strong predictor of detrimental health outcomes in older adults (Franklin, Brinks et al. 2015). There is an emerging concept of the physiological reserve in the elderly which relates to the difference between the maximum physical or mental capacity and the minimum necessary to perform daily activities, such as walking (Arnett, Laity et al. 2008, Wert, Brach et al. 2013, Schrack, Zipunnikov et al. 2014). As the physiological reserve reduces, and energy cost of activity increases, the adaptive process to conserve energy takes place which collectively results in the onset of fatigue and minimisation of unnecessary movement (Schrack, Simonsick et al. 2010, González, Cofré et al. 2016).

As it relates to diet, there are many nutritional aspects which enhance functional independence and longevity, specifically in the elderly (Leslie and Hankey 2015). Proportions of macronutrients in the diet, quality of fat sources, as well as key nutrients such as calcium, vitamin D and adequate protein intake have received recognition for their integrative role in protection from coronary heart

Notwithstanding the obvious and accepted role of protein to slow the rate of loss of muscle mass (Murphy, Oikawa et al. 2016), population based research indicates that diets high in polyunsaturated fatty acids, such as the Mediterranean diet reduce the risk of all-cause and cause-specific mortality (Kromhout, Bosschieter et al. 1985, Burr, Fehily et al. 1989, Siscovick, Raghunathan et al. 1995, Knoops, de Groot et al. 2004), and are associated with a slower decline in mobility and walking speed over time in the elderly (Abbatecola, Cherubini et al. 2009, Milaneschi, Bandinelli et al. 2011, Shahar, Houston et al. 2012, Frison, Boirie et al. 2015).

The findings of reduced all-cause mortality (Burr, Fehily et al. 1989, Mozaffarian, Lemaitre et al. 2013), primary cardiac arrest (Siscovick, Raghunathan et al. 1995, Mozaffarian and Wu 2011) and coronary heart disease mortality (Kromhout, Bosschieter et al. 1985) have been demonstrated with fish intakes of 2 meals per week. The current dietary recommendations of 2 fish meals per week are in line with the earlier findings (National Health and Medical Research Council 2013).

As well as the aforementioned prevention of cardiovascular disease, there is experimental evidence of the effects of fish oil that could pertain to some of the effects of aging on cardiac and muscle function. Fish oil supplementation has shown promise in the attenuation of the age related changes in muscle mass, strength and muscle protein metabolism (Smith, Atherton et al. 2011, Rodacki, Rodacki et al. 2012, Hutchins-Wiese, Kleppinger et al. 2013, Krzymińska-Siemaszko, Czepulis et
al. 2015, Smith, Julliand et al. 2015, Da Boit, Sibson et al. 2017). However, these studies have supplemented at therapeutic doses with EPA-rich fish oil capsules so it is not known whether the findings from these studies are achievable through dietary intake of 1-2 fish meals per week.

Evidence suggests that the long chain omega-3 polyunsaturated fatty acid (LC n-3 PUFA), docosahexaenoic acid (DHA), incorporates into human heart and skeletal muscle (Metcalf, James et al. 2007, McGlory, Galloway et al. 2014) and incorporation of DHA leads to positive changes within those tissues, which may benefit aging adults. In animal models, low doses of LC n-3 PUFA, in particular DHA, significantly incorporate into myocardial and muscle membranes, suggesting that membrane incorporation can occur through fish oil doses approximating 2 fish meals per week (Slee, McLennan et al. 2010, Henry, Peoples et al. 2015). Supplementing DHA-rich fish oil capsules in humans at 2 grams per day significantly incorporates into red blood cells, reflective of heart and skeletal muscle membranes (Macartney, Hingley et al. 2014, Hingley, Macartney et al. 2017), thus supporting animal evidence and demonstrating that incorporation into membranes is possible through DHA-rich fish oil approximating 2 fish meals per week.

This project combined a small cohort study and a randomised control trial to examine the potential benefits of LC n-3 PUFA in aging physiology. A small cohort study first explored the physiology of aging and in particular the energy cost of walking in a group of adults aged 60-85 years. In those identified as deficient in LC n-3 PUFA, a randomised control trial aimed to increase the omega-3 status using a dietary achievable dose of high-DHA tuna fish oil capsules to improve physical function, including walking speed, underpinned by the modulation of oxygen consumption and cardiovascular function.
1.2 Functional decline with age

1.2.1 Sarcopenia and declining strength

Sarcopenia is characterised by a progressive loss of muscle mass and strength as an individual ages (Cruz-Jentoft, Baeyens et al. 2010), which accelerates beyond the age of 70 years (Ganse, Ganse et al. 2018). Sarcopenia is associated with increased mortality (Landi, Cruz-Jentoft et al. 2013), increased falls risk (Tanimoto, Watanabe et al. 2014), and reduced quality of life (Beaudart, Reginster et al. 2015, Trombetti, Reid et al. 2016). Currently, slowing sarcopenia is a primary focus for reducing disability, reducing mortality, increasing mobility and quality of life in older aged adults.

Lifestyle interventions which include modifying nutrition and physical activity behaviours have an important role in slowing the progression of age related muscle and strength loss (Witard, McGlory et al. 2016). Physical activity interventions which focus on incorporating higher intensities of exercise such as resistance training improves muscle strength, balance, endurance, and time to fatigue (Ferketich, Kirby et al. 1998, Ferreira, Sherrington et al. 2012). With respect to nutritional intervention there has been a focus on protein and energy intake to combat nutritional frailty through counteracting ‘anabolic resistance’ with age (Breen and Phillips 2011, Moore, Churchward-Venne et al. 2015). Strategies which have addressed the quantity and frequency of protein consumption, and more specifically leucine-rich protein sources, along with kilojoule-rich supplements have produced promising results in the attenuation of skeletal muscle loss and strength loss, as well as the augmentation of muscle protein synthesis in older adults, especially when coupled with physical activity (Turic, Gordon et al. 1998, Paddon-Jones and Rasmussen 2009, Moore 2014, Moore, Churchward-Venne et al. 2015, Paddon-Jones, Campbell et al. 2015, Murphy, Oikawa et al. 2016, Murphy, Saddler et al. 2016). Whilst, with merit, muscle size and
strength share an important role in attenuating sarcopenia, relatively little attention has been given to the physiological cost of performing exercise in the aged adult, despite compelling evidence that oxygen efficiency of heart and skeletal muscle are linked to exercise related fatigue (Schrack, Simonsick et al. 2010). Recent research emphasising the concept of oxygen modulation, primarily displayed through walking speed now provides new potential to address declining function in older age (Schrack, Zipunnikov et al. 2016).

1.2.2 Cardiovascular decrements during aging

With age there are declines in cardiovascular system performance which includes changes in its structure, function and its ability to handle physiological stress such as ischemia (Strait and Lakatta 2012). The structural changes that occur include thickening of the left ventricular wall and arterial stiffness, which negatively influences the heart’s contractile efficiency (Gerstenblith, Frederiksen et al. 1977, Grodzicki, Michalewicz et al. 1998). The accelerated decline in cardiovascular function, and more specifically, aerobic capacity that is associated with age has wide reaching ramifications in older life (Fleg, Morrell et al. 2005). Functional independence of an older adult depends heavily on their ability to perform activities of daily living at the lowest physiological cost and perceived effort of activity. When the physiological cost of walking, expressed as volume of oxygen consumed per heartbeat, is reduced it leads to higher engagement of free-living physical activity and less sedentary behaviours (Carter, Hunter et al. 2018). Ischaemic preconditioning refers to the heart’s intrinsic process of resisting damage and limiting blood and oxygen supply to organ systems during ischemia. It is noted that with age, the hearts intrinsic ability to resist damage is reduced (Juhaszova, Rabuel et al. 2005). Nutritional strategies which incorporate omega-3 fatty acids have demonstrated a similar preconditioning effect on the heart (Abdukeyum, Owen et al.
2008), which may be of importance due to the decline in the heart’s own intrinsic ability seen with age.

There are a number of clinically attainable measures directly pertaining to the heart such as resting heart rate and heart rate recovery following exercise which are now understood to have a strong association with future disease prediction. Resting heart rate is a useful heart measure due to its ease of use in clinical settings and its strong prognostic importance in mortality and cardiovascular risk (Cooney, Vartiainen et al. 2010, Saxena, Minton et al. 2013). In elderly populations, an elevated heart rate is a predictor of cardiovascular death, whereas a lower heart rate is related to better health outcomes (Palatini, Casiglia et al. 1999). Whilst resting heart rate provides valuable information about the cardiovascular system, its collection may be influenced by external factors relating to environmental stimuli such as room light (Smolders, de Kort et al. 2012), body position (Watanabe, Reece et al. 2007) and psychological factors (Mancia, Bertinieri et al. 1983). Therefore, a measure of heart rate which is collected in more controlled manner, such as during night-time sleep, appears to serve as a stronger measure in determining mortality and cardiovascular risk in elderly populations (Johansen, Olsen et al. 2013). Therefore, in order to comprehensively assess the heart rate profile, one primary objective of the current study was to collect ECG quality depolarisation across a range of conditions pertaining to rest, exercise and recovery.
1.2.3  *Oxygen cost of exercise and the impact of aging*

The de-adaptation of oxygen delivery and oxygen utilisation systems with age has an important role in physical activity output due to the role that oxygen availability and uptake has as a regulator of developed tension and fatigue (Hogan, Arthur et al. 1992, Burtscher 2013). The energy cost of walking increases with age (Wert, Brach et al. 2013), leaving a lower energy reserve for additional activities throughout the day whereby the “physical cliff” occurs, characterised by a rapid acceleration in the decline in physical activity across 24 hours that occurs from mid to late life (Schrack, Zipunnikov et al. 2014). Brought on initially by the heightened physiological cost of activity, this trend for lower activity towards the end of the day results from higher self-perceived effort and fatigue to activity and the subsequent avoidance of further activity to conserve energy (Fleg, Morrell et al. 2005).

In older adults, preferred walking speeds of 0.8 meters per second (m/s) predicts mortality and adverse health outcomes (Abellan van Kan, Rolland et al. 2009). Walking speeds over 1.0m/s suggests better than average life expectancy and improvements in activities of daily living, with speeds over 1.2m/s suggesting exceptional life expectancy (Abellan van Kan, Rolland et al. 2009, Studenski, Perera et al. 2011). This has been confirmed in the Invecchiare in Chianti (InCHIANTI) study which reported physical inactivity and walking speed as the frailty criteria showing the strongest associations with muscle density and muscle mass (Cesari, Leeuwenburgh et al. 2006). 75% of Australian adults aged over 65 years old report being insufficiently active (did not meet the minimum recommended physical activity guidelines) and therefore are at a heightened risk of physical function declines (Australian Bureau of Statistics 2015). It is established that older adults who are physically active and partake in habitual exercise have a slower decline in physical function (Westerterp 2000, Riebe, Blissmer et al. 2009). Taken together, these findings highlight
the importance of maintaining physical activity and walking speeds as an individual ages in order to combat declines in muscle mass and quality of life.

Measuring walking speed is a cost effective, clinically relevant and practical field test which is suggested for routine measurement as an additional vital sign in older adults (Middleton, Fritz et al. 2015). Walking speed is easily administered by health professionals and has strong predictive power across a range of adverse health events in aged populations such as cardiovascular events (Matsuzawa, Konishi et al. 2013) and cardiovascular mortality (Dumurgier, Elbaz et al. 2009), frailty (Castell, Sanchez et al. 2013), falls (Montero-Odasso, Schapira et al. 2005) and survival rates (Studenski, Perera et al. 2011), as well as being a good marker of functional status (Verghese, Wang et al. 2011). Therefore, the current study has assessed the physiology of walking in older adults and the potential to modulate this by increasing DHA intake via a DHA-rich fish oil supplement at a dose that is equivalent to consuming 2 fatty fish meals per week.

1.3 The influence of diet and nutrition behaviours on health and longevity
Diet behaviors, diet quality and the composition of macronutrients within the diet are known to influence health outcomes and life expectancy (Wirt and Collins 2009). Currently, the country with the highest life expectancy is Japan (World Health Organization 2018). When assessing Japanese dietary habits there are key elements which may influence their longevity. Japanese diets comprise higher fish consumption relative to western culture, as well as lower energy density of food sources relative to nutrients (Ministry of Health Labour and Welfare Japan 2014, Murakami, Livingstone et al. 2017). Adherence to the Japanese dietary guidelines resulted in lower overall mortality and cardiovascular mortality (Kurotani, Akter et al. 2016). Diets such as the Mediterranean diet which share similar characteristics with Japanese culture, including high fish
consumption and intake of nutrient rich foods, reduce the risk of all-cause and cause-specific mortality (Burr, Fehily et al. 1989, Knoops, de Groot et al. 2004). The InCHIANTI study, a large population-based study of older Italians, determined that, upon measurement of plasma LC n-3 PUFA concentration, an indicator of fish consumption, participants with the highest levels of plasma LC n-3 PUFA were also deemed to have the highest functional status (Abbatecola, Cherubini et al. 2009). Another population study conducted in older adults, The Three-City-Bordeaux Study, also found a positive association between walking speed and plasma LC n-3 PUFA concentrations, with particular importance to the higher proportion of DHA (Frison, Boirie et al. 2015). Therefore, it is plausible that a diet that allows provision for LC n-3 PUFA naturally derived from fish intake, has a mechanistic basis, such as modulation of skeletal muscle and cardiac function achieved by cell membrane incorporation of specific fatty acids to improve function of contractile tissue, such as heart and skeletal muscle (McLennan, Owen et al. 2007, Peoples and McLennan 2010). This warrants further investigation and is a primary focus of this thesis.

1.4 Fish oil providing long-chain omega-3 polyunsaturated fatty acids

Dietary fish oil incorporates long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) into cardiac (Metcalf, James et al. 2007) and skeletal muscle membranes (Andersson, Nalsen et al. 2002, McGlory, Galloway et al. 2014), with a dominance for DHA incorporation into fast twitch (type II) fibres (Henry, Peoples et al. 2015). Incorporation of LC n-3 PUFA into cardiac muscle cells reduces heart rate and improves myocardial oxygen efficiency (Pepe and McLennan 2002, McLennan, Owen et al. 2007, Hidayat, Yang et al. 2017). In animals, feeding LC n-3 PUFA reduces skeletal muscle fatigue and improves skeletal muscle oxygen consumption (Peoples and McLennan 2010, Peoples and McLennan 2014). These effects, initially observed after higher
doses, have been replicated with dietary achievable doses of fish oil in animals (Henry, Peoples et al. 2015) and young adults (Hingley, Macartney et al. 2017). DHA enrichment, following low doses of fish oil (2g/day) improves heart rate recovery after intense exercise (Macartney, Hingley et al. 2014) and lowers the oxygen cost of exercise in trained male participants (Hingley, Macartney et al. 2017).

1.4.1 Fish consumption: Current intakes and trends

The suggested dietary target (SDT) from The National Health and Medical Research Council (NHMRC) for LC n-3 PUFA is 430mg/d for females and 610mg/d for males (National Health and Medical Research Council 2006). These suggested targets closely relate to a fish intake of 2 fish meals per week (providing LC n-3 PUFA of approximately 430-570 mg/d) (National Health and Medical Research Council 2006). However, the suggested dietary targets are not based on a physiological or nutrition effect but rather on the 90th percentile of usual intake.

In Australia, the mean LC n-3 PUFA intake of older adults (>65 years) has increased more than 2-fold between 1995 and 2012 (Meyer 2016). Current mean LC n-3 PUFA intakes for adults over 65 years are 494mg/d for females and 441mg/d for males. Despite an increase in the mean intake of LC n-3 PUFA, the current median LC n-3 PUFA intake in older adults is well below the SDT. The median LC n-3 PUFA intake in older adults is approximately 120mg/d, and overall less than 20% of Australians are meeting the SDT (Meyer 2016). When dietary guidelines which include adequate fish consumption are adhered to, such as in Japan, there is a clear benefit in reducing mortality and more specifically, cardiovascular mortality rates (Kurotani, Akter et al. 2016). Therefore, it is possible that supplemental fish oil which is in line with dietary achievable amounts may benefit cardiovascular outcomes in older adults.
1.4.2  Fish oil and cardioprotection

Fish oil, providing omega-3 fatty acids have been a topic of debate regarding its efficacy and role in cardiovascular disease risk and mortality. Strong evidence suggests that when clinical trials are appropriately designed, that is, to have strict exclusion criteria, be powered to detect changes in primary outcome measures and to have accurate measures of circulating LC n-3 PUFA before and after the trial, that there is a clear relationship between fish intake and cardio-protection. Early population-based and observation studies established links between fish consumption and reduced risk of cardiovascular related health issues (Bang, Dyerberg et al. 1971, Kromhout, Bosschieter et al. 1985, Siscovick, Raghunathan et al. 1995). Evolutionary work in the 1970’s set the hypothesis that the high consumption of seafood consumed by the Greenlandic Inuit’s was responsible for their low cardiovascular disease occurrence (Bang, Dyerberg et al. 1971). There is a recurring notion throughout early studies that 1-2 fish meals per week are able to improve cardiovascular outcomes. Kromhout et al., (1985) established an inverse, dose-response relationship between fish consumption and death from coronary heart disease after a 20 year follow up. Researchers determined that fish consumed at a frequency of 1-2 fish meals per week has preventative power of over 50% for coronary heart disease mortality. Siscovick et al., (1995) supported these findings, whereby fish consumption at a frequency of 1-2 fish meals per week in their study was associated with a 50% reduction in primary cardiac arrest. Furthermore, and of particular importance to the current study, in middle-aged and elderly men, consumption of fish weekly reduces the risk of fatal myocardial infarction (Yuan, Ross et al. 2001) and cardiovascular risk factors when fish is consumed twice per week (Mizushima, Moriguchi et al. 1997). The earlier findings of the cardio-protective role of LC n-3 PUFA from fatty fish have been supported in larger population studies such as The DART Trial which established that 2 fish meals per week reduces the risk of all-cause mortality (Burr, Fehily et al. 1989), and the GISSI-Prevenzione Trial, which included 11,324...
participants who had survived recent myocardial infarction and when given LC n-3 PUFA had decreased rates of death, non-fatal myocardial infarction and stroke over 3.5 years follow up (Valagussa 1999).

It has been demonstrated that a low intake of fish oil is sufficient to provide LC n-3 PUFA incorporation in myocardial membranes in rats with a preference for DHA incorporation (Slee, McLennan et al. 2010) and that this leads to altered myocardial effects such as an anti-arrhythmic action on the heart (McLennan, Abeywardena et al. 1988, Pepe and McLennan 1996, Pepe and McLennan 2002). Incorporation of LC n-3 PUFA into cardiac muscle cells reduces heart rate and improves myocardial oxygen efficiency even in isolated hearts (Pepe and McLennan 2002, McLennan, Owen et al. 2007). These findings provide evidence for a direct cardioprotective effect on the heart as a result of LC n-3 PUFA incorporation into myocardial membranes. In humans, DHA selectively lowers heart rate in healthy men (Grimsgaard, Bonaa et al. 1998) and in mildly hyperlipidemic men (Mori, Bao et al. 1999), and offers a nutritional approach to modifying cardiovascular disease risk, specifically pertaining to cardiac origin.

1.4.3  Fish oil and sarcopenia

Fish oil has gained attention as a key nutrient in a range of age related health issues, including bone health, cognitive decline, and muscular function (Molfino, Gioia et al. 2014). Attempts at combatting sarcopenia with fish oil supplementation have targeted the known declines in muscle strength and size, as well as anabolic resistance with age (Smith, Atherton et al. 2011, Rodacki, Rodacki et al. 2012, Hutchins-Wiese, Kleppinger et al. 2013, Di Girolamo, Situlin et al. 2014, Krzymińska-Siemaszko, Czepulis et al. 2015, Smith, Julliand et al. 2015, Da Boit, Sibson et al. 2017, Alfaddagh, Elajami et al. 2018).
Smith et al., (2011) worked with a small sample of older adults (n=15) to investigate the effects of a high-EPA, high-dose fish oil supplement (4g/day) taken for 8 weeks on muscle protein synthesis. Researchers found that basal muscle protein synthesis was not influenced by fish oil supplementation, however fish oil did augment the anabolic response from the hyperaminoacidemia-hyperinsulinemia induced increase in the rate of muscle protein synthesis. This study established that fish oil supplementation, albeit at a dose not likely reproduced through dietary fish intake alone, incorporated significantly into muscle phospholipids in older adults.

The role of physical activity, and more importantly strength training for the prevention of falls, attenuation of muscle mass and strength loss, as well as improved quality of life in the elderly is well established (Ferketich, Kirby et al. 1998, Persch, Ugrinowitsch et al. 2009, Mayer, Scharhag-Rosenberger et al. 2011, Ferreira, Sherrington et al. 2012). Building on the hypothesis from Smith et al., (2011) that fish oil has anabolic signalling properties in older adults, Rodacki et al., (2012) combined fish oil supplementation with strength training in older women to determine whether fish oil may augment the benefits of strength training. In contrast to Smith et al., (2011), this study used a dietary achievable dose of fish oil taken over 12 weeks, however, this study included only women, was not placebo-controlled and all groups performed strength training throughout the study. The fish oil group achieved a significant incorporation of EPA and DHA into plasma phospholipids. The increase in EPA was twice as much as DHA. This was likely due to the EPA-rich fish oil capsule used in the study. Fish oil was effective at increasing peak torque and rate of torque development in the knee flexor/extensor and plantar/dorsiflexor above what was seen in the group who performed strength training alone. Given that greater rate of torque development in the
knee flexor muscle group is associated with less falls in the elderly (Bento, Pereira et al. 2010), this suggests that LC n-3 PUFA from fish oil has the potential to decrease falls risk with age when coupled with strength training. The participants in this study were all sedentary females at baseline so the findings may not extend to older populations with a higher activity level and which include males. In fact, Da Boit et al., (2017) sought to determine whether there were sex differences in how a high-dose, high-EPA fish oil supplement may augment adaptations to resistance training in an active group of older males and females. Fish oil does indeed seem to increase resistance training adaptations to a greater extent in females compared to males, as seen by Da Boit et al., (2017), where only the female group receiving fish oil increased peak torque and muscle quality (strength generated per unit of muscle mass). However, it should be noted that there was no measure of dietary intake. The importance of dietary intake, especially when combined with resistance training in older adults is well established and therefore consideration of controlling dietary intake should be accounted for in light of the reported findings (Mori and Tokuda 2016).

In a later study by Smith et al., (2015), participants were supplemented with the same high-dose (4g/day), high-EPA fish oil capsule as used in their 2011 study, however now researchers investigated whether 3 months or 6 months of fish oil supplementation improves muscle mass and muscle strength in older adults. Interestingly, fish oil supplementation only influenced muscle strength after 6 months, whereas it was unchanged at the 3 month time point. In addition, participants in the control group decreased their muscle thigh volume and muscle strength to a similar extent that the fish oil group increased their muscle thigh volume and muscle strength. Given that participants in this study were healthy and active older adults it is not known why this occurred. Since there was no measure of physical activity or dietary intake before or after the
intervention, it is difficult to know whether changes in activity level or dietary intake may have influenced these findings. When fish oil was supplemented at a dose reproduceable through dietary intake of approximately 2 fish meals per week (~2g/day), Krzyninska-Siemaszko et al., (2015) did not find any changes in muscle mass or muscle strength in older adults with decreased muscle mass after 12 weeks. This study, however, did not measure circulating levels of EPA or DHA before or after supplementation. Without a measure of baseline and post-supplement levels of EPA and DHA, either within muscle tissue or circulating levels in plasma or red blood cells, it is impossible to establish whether participants had the same levels of LC n-3 PUFA at baseline, or whether the intervention caused a change post supplementation. Nonetheless, similar results were found when blood levels of EPA and DHA were measured by Hutchins-Wiese et al., (2013) who supplemented older females who met pre-frail or frail criteria with a low dose (2g/day) fish oil supplement and not find any changes in strength measures as a result of fish oil supplementation. Taken together, it appears that high-dose fish oil primarily augments strength-related increases only when combined with resistance training and the effect is greater in females.

To date, research involving omega-3 fatty acids to combat sarcopenia and improve muscle function in the elderly have used EPA-rich capsules which do not reflect fatty acid profiles of fresh fish, which is higher in DHA (Smith, Atherton et al. 2011, Rodacki, Rodacki et al. 2012, Hutchins-Wiese, Kleppinger et al. 2013, Krzyninska-Siemaszko, Czepulis et al. 2015, Smith, Julliand et al. 2015, Da Boit, Sibson et al. 2017, Alfaddagh, Elajami et al. 2018), have used doses which exceed amounts achievable through food alone (Smith, Atherton et al. 2011, Smith, Julliand et al. 2015, Da Boit, Sibson et al. 2017, Alfaddagh, Elajami et al. 2018), or did not measure LC n-3 PUFA within muscle phospholipids or blood to ensure participants start with low levels of omega-3 fatty
acids, to match intervention and control groups at baseline and to establish a change in muscle or blood levels as a result of fish oil supplementation (Krzymińska-Siemaszko, Czepulis et al. 2015, Alfaddagh, Elajami et al. 2018).

A range of factors need to be considered when conducting research involving omega-3 fatty acids, including doses that are high enough to see an effect in the outcome measures within a manageable population sample, but also achievable through diet. Additionally, a measurement of baseline blood fatty acids to ensure participants start with low circulating omega-3 concentrations, and to match control and intervention groups to ensure no significant differences at baseline. The present study used a finger prick method to collect blood for determination of the omega-3 index in red blood cells (Harris and Von Schacky 2004). The omega-3 index is a measure of the proportion of EPA plus DHA in red blood cell membranes and a low omega-3 index has been proposed as a cardiovascular risk factor. The omega-3 index is inversely associated with risk for coronary heart disease (Harris and Von Schacky 2004) and there are now proposed target ranges which may further classify risk. An omega-3 index value over under 4% is suggested be high risk, between 4% and 8% indicates an intermediate risk, whereas over 8% suggests low risk and provides the greatest cardio-protection (Harris 2008). To date, little work has been done to investigate whether the omega-3 index improves cardiovascular measures not relating to mortality rates, such as heart rate. Elevating the omega-3 index lowers heart rate in depressed patients with coronary heart disease and in cardiac transplant patients (Harris, Gonzales et al. 2006, Carney, Freedland et al. 2010), however it is unclear whether an elevated omega-3 index in older adults has a similar effect of lowering heart rate. As it relates to older adults, the omega-3 index is inversely associated with performance-based test results (Fougere, de Souto Barreto et al. 2018), however little work has
been done on cardiovascular outcomes such as heart rate in this population. Therefore, the current study will attempt to determine whether elevating the omega-3 index using a dietary achievable dose of fish oil in older adults will improve heart rate during rest, exercise and night time sleep.

1.4.4 *Fish oil and oxygen efficiency*

The role of omega-3 fatty acids in oxygen modulation within skeletal muscle (Peoples and McLennan 2010, Peoples and McLennan 2014, Henry, Peoples et al. 2015) provides a nutritional target for reducing oxygen cost of exercise, freeing up energy reserve and reducing fatigue. In humans, Logan *et al.*, (2015) dealt with functional measures such as the timed up and go test in older age females, with improvements being found in the group supplemented with fish oil. Despite showing improvements in physical function outcomes, the dosage of 5g/d of fish oil used in the Logan *et al.*, (2015) study is difficult to attain through diet alone and would require supplementation. Therefore, the current study adopted a fish oil intake of 2g/d of DHA-rich tuna fish oil capsules (delivering 140 mg of EPA and 560 mg of DHA per day) which translates to approximately 2 fish meals per week in order to determine if dietary achievable fish intakes may improve physical function outcomes.

Of particular importance to aging, DHA incorporates greater relative concentrations in fast-twitch muscle fibres (type II) compared to slow twitch muscle fibres (type I) (Henry, Peoples et al. 2015). There is a general shift towards lower levels of planned physical activity and higher levels of sedentary behaviour across all age groups in Australia. This is particularly evident in adults aged 65 and over, showing marked declines in overall and vigorous physical activity (Australian Bureau of Statistics 2015). Increased levels of moderate to vigorous physical activity significantly counteracts sarcopenia (Mijnarends, Koster et al. 2016). The reduction of vigorous activities with
age may perhaps be due to the dominant atrophy of fast-twitch (type II) muscle fibres relative to slow-twitch (type I) muscle fibres (Deschenes 2004). Type II muscle fibres are responsible for engagement in higher intensities of activity. Using a DHA-rich fish oil capsule, this study proposed that the incorporation of DHA into skeletal muscle will reduce fatigue and energy costs of walking, leading to a greater energy reserve to improve participation in physical activity.

1.5 Aims and hypothesis

To date, improvements in cardiac and skeletal muscle function have been investigated in animal models or in healthy, young adults. Due to the low doses of fish oil required to significantly improve membrane DHA incorporation in animals (Slee, McLennan et al. 2010, Henry, Peoples et al. 2015) and healthy younger adults (Macartney, Hingley et al. 2014, Hingley, Macartney et al. 2017), this study tested the feasibility that low dose DHA-rich fish oil can elevate the omega-3 index in older adults and therefore improve heart rate profiles at rest and during exercise and recovery. Furthermore, as a result of an elevated omega-3 index and the expected changes to skeletal muscle membranes it is feasible that the oxygen cost of exercise will be lowered and physical function improved.

Aim 1: Determine whether a low dose DHA-rich fish oil supplement would improve the omega-3 index in older adults over the course of 16 weeks. The dose delivered LC n-3 PUFA DHA (560mg/day) and EPA (140mg/day) and was equivalent to consuming 1-2 fish meals per week.

Hypothesis 1: It was hypothesised that a DHA-rich fish oil supplement would significantly modify the whole blood and specifically the erythrocyte membranes to reflect LC n-3 PUFA DHA in the diet and therefore elevate the omega-3 index.
Aim 2: Investigate whether an increased omega-3 index will improve heart rate profiles at rest and during exercise and recovery in older adults.

Hypothesis 2: Due to the established role of omega-3 fatty acids in cardiac protection, it was hypothesised that an elevated omega-3 index would lower heart rate, independent of autonomic nervous system function, at rest and during exercise and recovery in older adults.

Aim 3: Determine if an elevated omega-3 index modifies the oxygen cost of exercise and improves physical function, in particular walking speed, in older adults.

Hypothesis 3: It was hypothesised that DHA-rich fish oil supplementation would lower the oxygen cost of exercise and improve physical function.
2. General Methods

2.1 Study design

This study was a double-blind design with placebo control. Older adults, aged 60-85 years were supplemented for 16 weeks with either 2 grams per day of DHA-rich tuna fish oil or placebo oil (Sunola). The DHA-rich tuna fish oil provided long chain omega-3 fatty acids achievable in the human diet via the consumption of 2 fatty fish meals per week. Physiological status and physical function were measured at baseline and following the 16 weeks of supplementation. Approval for ethics was applied for in April 2016 from the University of Wollongong Human Research Ethics Committee and approved in July 2016 (HE16/169).

2.2 Experimental overview

Selected participants attended the University of Wollongong’s Exercise Physiology Patient Simulation Laboratory for baseline (week 0) and post-supplement (week 16) assessments. Each visit was approximately 2.5 hours in duration. Baseline testing included physiological and physical function assessments and ambulatory heart rate monitoring. Participants who reported a fish consumption of 2 fish meals or more per week were excluded at baseline. Participants meeting all the inclusion criteria were then randomly allocated to either a control (Sunola oil) or intervention (DHA-rich fish oil) group delivered as 1 gram capsules. Each participant consumed 2 grams of oil per day over the duration of the 16 weeks. The physiological and physical function assessments and ambulatory heart rate monitoring performed at baseline were repeated after 16 weeks. The experimental overview is shown in figure 2.1.
Abbreviations: PUFA, polyunsaturated fatty acid; ECG, electrocardiogram.
2.3 Participant recruitment and selection

Physically active participants, aged between 60-85 years, were required for the study in order to complete the range of physical assessments. Participants were recruited from local physical recreation facilities (including tennis clubs, lawn bowling clubs, cycling clubs and walking clubs), and the University of the Third Age within the Illawarra region of Australia. Participants were able to ambulate without an assistive device.

Each participant completed a series of questionnaires which were:

- The Physical Activity Readiness Questionnaire (PAR-Q) to determine whether participants were physically able to perform physical activity and to limit adverse events,
- The International Physical Activity Questionnaire – Elderly (Hurtig-Wennlof, Hagstromer et al. 2010) to establish their baseline physical activity level and to enable detection of physical activity change by the end of the intervention;
- The Pittsburgh Fatigability Scale (Glynn, Santanasto et al. 2015) to understand participants’ self-perceived mental and physical fatigue, as well as any change by the end of the intervention;
- The Mini-Nutritional Assessment – Short Form (MNA®-SF) (Vellas, Guigoz et al. 1999) to determine whether participant’s were at risk of being malnourished (reporting a score <12).

Participants were also screened for medications that directly affect the cardiovascular system (for example, Beta Blockers).

In addition, participants performed a pulmonary function test using a hand held spirometry device (ParvoMedics, USA). This assessment was conducted both before and after the supplementation period. The pulmonary function test focused on the main reported determinants of respiratory fitness and health; forced expiratory volume in 1 second (FEV₁) (L) which measured the amount
of air that was forcefully exhaled within the first second of the test and forced vital capacity (FVC) (L) which measured the total amount of air exhaled during the test (Sin, Jones et al. 2004, Sin, Wu et al. 2005). Participants were initially familiarised with the procedure. Each participant was instructed to perform the test twice, with a 1 minute break between attempts. On each attempt they were instructed to take a full breath in, to fill the lungs, and then rapidly exhale as quickly and forcefully as possible. If there was a greater than 10% difference between force expiratory volume, they were provided with a third attempt. If this was the case, the best attempt was taken as the final result. The recruitment and screening process is detailed, with reasons for exclusion at each step in figure 2.2.

There were a number of exclusion criteria that was applied to ensure physically active participants who also described as having low intakes of long chain omega-3 fatty acids. For the former, participants were excluded if they had, (i) difficulty performing daily living activities, (ii) history of active cancer in last 3 years, (iii) recent or planned major surgery, (iv) currently smoking, (v) uncontrolled hypertension, (vi) dyslipidaemia, (vii) Body Mass Index (BMI) <18 or >32, or (viii) lung health indicative of COPD. Reference criteria of >70% FEV₁/FVC was used (Hardie, Buist et al. 2002) and participants who did not meet this criteria were excluded. For the latter, participants were also excluded if they reported using omega-3 and or fish oil supplements in the last 6 months or, based upon preliminary screening using the food frequency questionnaire (FFQ) specific for omega-3 and omega-6 food sources (Swierk, Williams et al. 2011) they were identified as consistently consuming up to 2 fish meals per week. Ultimately, red blood cell omega-3 index (EPA+DHA) was the tool used to determine omega-3 status.
Figure 2.2. Participant screening, recruitment and completion flow chart.

Details of the withdrawals and exclusion are included on the far right hand side of the figure.
2.4 Participant allocation to groups

The participants were matched for characteristics such as age, sex and BMI as well as several primary outcome measures. These included 400m walk time (m/s), resting heart rate (bpm), and the omega-3 index (percentage of EPA and DHA in red blood cells).

In terms of allocation to supplement, neither the participants nor the research student was informed of the specific group allocation, thus making the study double blinded. The primary supervisor allocated the capsules to each of the participants and the capsules were provided in colour-coded blister packs. Instructions for taking the capsules were provided in the package and this included consuming with a meal.

2.5 Dietary supplementation

The control group were supplemented with 2 grams per day of Sunola oil which delivered 160mg per day of the n-6 PUFA linoleic acid (18:2n-6) and 1620mg per day of monounsaturated oleic acid (18:1n-9). The intervention group (FO) were supplemented with 2 grams per day of DHA-rich, tuna fish oil capsules, which delivered 140mg of EPA and 560mg of DHA per day (700mg per day total). Fatty acid composition tables are provided in table 2.1 for Sunola Oil and DHA-rich tuna fish oil.
Table 2.1. Major fatty acid percentage composition of the dietary supplements used in the study

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Sunola Oil (Control)</th>
<th>Fish Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>∑ Saturated Fatty acids</td>
<td>9.0</td>
<td>31.7</td>
</tr>
<tr>
<td>∑ Mono-unsaturated Fatty acids</td>
<td>81.0</td>
<td>21.4</td>
</tr>
<tr>
<td>∑ Minor Fatty acids</td>
<td>-</td>
<td>6.1</td>
</tr>
<tr>
<td>∑ Omega-6 PUFA</td>
<td>8.0</td>
<td>6.3</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>-</td>
<td>0.6</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>-</td>
<td>5.3</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>-</td>
<td>25.0</td>
</tr>
<tr>
<td>∑ Omega-3 PUFA</td>
<td>-</td>
<td>34.6</td>
</tr>
</tbody>
</table>

Abbreviations: 18:3n-3, *alpha*-linoleic acid; 20:5n-3, *eicosapentaenoic acid*; 22:5n-3, *docosapentaenoic acid*; 22:6n-3, *docosahexaenoic acid*. Fatty acid composition provided by Clover Corporation Ltd. Minor fatty acids include fatty acids which were unidentified from the standard.
3. Laboratory assessments

3.1 Overview

The following assessments were completed both before and after the supplementation period of 16 weeks. On arrival at the Exercise Physiology Laboratory, participant anthropometrics were initially recorded according to International Standards for Anthropometrics. A 3-lead electrocardiogram was attached to their chest for electrocardiograph screening by an Accredited Exercise Physiologist (Australian Exercise and Sport Science Association). Resting heart rate and blood pressure were collected, in a supine position over 5 minutes duration, prior to physical assessments and a muscle oxygen monitor was secured to their gastrocnemius muscle (medial head). Upon meeting the pre-screening requirements (see exclusion criteria, section 1.3) they then began their dietary assessments and the senior fitness tests which included grip strength, bicep curl repetitions, sit to stand repetitions, timed up and go, and walking speed measured over 20m and 400m. Finally, participants completed a submaximal treadmill test, followed by a controlled recovery phase. The entire assessment took approximately 2.5 hours.

3.2 Anthropometry and participant preparation

Participant’s body mass and height were measured using anthropometric scales (SECA875 flat scale, SECA, United Kingdom) and stadiometer (SECA217 Stable Stadiometer, SECA, United Kingdom) with the participant lightly clad and shoes off. A Polar heart rate strap (Model RS800CS, Polar, Kempele, Finland) was placed across the chest of the participants and remained on throughout the duration of their visit in order to continually collect recordings of heart rate. Blood pressure was recorded on the left arm of the participant, in a supine position, using an OMRON home monitoring device (Model Omron IA2, Omron Healthcare Co, Kyoto, Japan).
Continuous wave near-infrared spectroscopy (NIRS) was used to determine relative changes in local muscle oxygen saturation (SmO2) during rest and exercise. A muscle oxygen monitor (MOXY Fortiori Design, Minnesota) was placed on the gastrocnemius muscle (medial head) of the dominant lower limb with black non-adhesive bandage tape placed on top of the monitor to prevent external light from influencing readings. An elastic Velcro wrap secured the monitor to the limb and prevented movement during activity.

3.3 Assessment of nutritional status and dietary intake

Nutritional status of participants was assessed using the Mini Nutritional Assessment – Short Form (MNA®-SF) which served as a screening instrument at baseline. The MNA®-SF is a valid tool for people over 65 years. It uses a point scoring system ranging from 0-14 to determine nutritional adequacy. In order for participants to continue in the study they needed to score between 12-14, deemed ‘normal nutrition status’. Dietary assessment involved an electronically administered combined food frequency questionnaire (FFQ) specific for omega-3 and omega-6 food sources (Swierk, Williams et al. 2011). The food frequency questionnaire took approximately 15-20 minutes to complete.

A 3-day food record was administered to participants at baseline and post supplementation. Participants were instructed to record and approximate the serving size of all food and beverages consumed for breakfast, lunch and dinner, including any snacks for 3 consecutive days which included 2 week days and 1 weekend day. Participants were instructed to maintain usual eating and drinking habits and were not required to weigh foods, but were asked to provide household measurements for foods consumed (i.e. cups, tablespoons). Food records were returned to the laboratory at their next visit where they were checked for accuracy and completion, in addition to
asking the participant further questions such as portion sizes and drinks consumed. Data was manually entered into FoodWorks 8 (Xyris Software, Australia) diet and nutrition analysis software. The Ausbrands 2016 and Ausfoods 2016 databases were used. Data was then exported to Microsoft Excel (2016) for analysis, which included determining the average daily intake of participants at baseline and post supplementation.

3.4 Senior fitness assessments

A battery of physical fitness tasks were performed before and after the supplementation period. The Senior Fitness Test is an accepted and validated combination of functional tasks that can be scored according to normative values (Rikli and Jones 1999).

3.4.1 30 second arm curl

Upper body strength and endurance was assessed with the 30 second arm curl test. The participant braced their upper (dominant) arm against their body whilst they curled the weight up and down. A 2kg weight was used for female participants and a 3kg weight was used for male participants. The total number of complete arm curls (up and down equal’s 1 curl) within 30 seconds was recorded.

3.4.2 Grip strength

Maximal isometric hand and forearm strength (kg) were assessed using a hand dynamometer and pinch dynamometer. For the hand grip strength test, participants were instructed to exert maximal force for 5 seconds using a hand dynamometer (SM-K3 series, Total Patient Care, Sydney, Australia). This was performed twice on both dominant and non-dominant arms and the highest value was recorded for analysis. Participants were instructed to perform a pinch grip strength test.
using the lateral/key punch method. Hand-held dynamometry and pinch test has good test-retest reliability and concurrent validity in older community dwelling persons (Abizanda, Navarro et al. 2012).

3.4.3 30 second Chair stand
The 30 second chair stand test assessed leg strength and endurance. Participants began in a seated position with their hands across their chest and were instructed to stand up with their knees fully locked out and hands still across their chest before returning to the seated position. This was repeated as many times as possible within 30 seconds. The total number of completed chair stands (up and down equals 1 stand) within 30 seconds was recorded.

3.4.4 Time up and go
The timed up and go test assessed the participant’s speed, agility and balance whilst moving. Participants were instructed to stand, walk to the cone (2 meters in front), turn around and walk back to a seated position. The time (seconds) between the initial seated position to the return to seated position was recorded. This test was performed twice and the fastest recording used for analysis.

3.5 Walking assessments
Walking speed was assessed under 3 conditions: i) self-selected walking speed over 20m, ii) walking rapidly over 20m, and iii) the 400m rapid walk test. The 20m walk protocols followed the design used by Shahar (2012). Participants were instructed to walk at their usual pace for 1 return lap (20m) between cones placed 10m apart and then as fast as they can for the same distance. For the 400m rapid walk test, participants walked between 2 distance markers placed 10m apart.
Participants walked as quickly as possible between cones placed 10m apart until they completed 400m in total. Their time (in minutes and seconds) of completion was recorded, in addition to time taken every 40m (seconds).

Following the 400m walk test, participants were immediately returned to a seated position for 2 minutes for heart rate recovery determination.

3.5.1 Experimental measures and analysis

3.5.1.1. Speed

Walking speed during the 400m walk test was calculated for each 40m lap (meter per second), in addition to the overall time to complete the 400m walk test.

3.5.1.2. Heart rate

Heart rate continuously measured on a beat to beat basis (ventricular depolarisation) during the 400m walk test was downloaded to the Polar Protrainer 5 software program (Finland). The files were then converted to text files which allowed them to be imported into Kubios heart rate variability program (Department of Physics of the University of Kuopio, Finland) where they were analysed. Heart rate was averaged on a lap by lap basis, in addition to an overall mean for the entire walk test.

Heart rate recovery following the 400m walk test was assessed as the beat reduction from their final heart rate during the 400m walk test and their heart rate at 1 minute post and 2 minutes post walk test.
3.5.1.3. **Muscle Oxygen**

Continuous wave near-infrared spectroscopy (NIRS) was used to determine relative changes in local muscle oxygen saturation (SmO2) during rest and exercise. The near-infrared spectroscopy device measured muscle tissue saturation by emitting near-infrared light (wavelength, 680-800nm) into the muscle tissue. Reflected near-infrared light from the tissue is received by 2 near-infrared spectroscopy detectors positioned 12.5 and 25mm away from the light source and allows accurate readings of muscle oxygenation through ~12mm of skin and adipose tissue. The near-infrared spectroscopy device used an algorithm created from a Monte Carlo model of light traveling through layers of tissue consisting of the epidermis, dermis, adipose and muscle tissue. The algorithm is designed for high sensitivity to the muscle layer and low sensitivity to everything else.

Muscle oxygen saturation was collected at 2 second intervals during the 400m walk. Near-infrared spectroscopy data files were downloaded as Microsoft Excel files. Resting tissue saturation index was calculated by averaging the final minute of the pre-exercise seated rest. Exercise tissue saturation was determined by averaging each 40m lap as well as the overall mean for the 400m walk test. A spreadsheet was created to calculate relative tissue saturation index for each lap by using the following equation,

**Equation 1:** \[
\left( \frac{\text{Exercising TSI} - \text{Resting TSI}}{\text{Resting TSI}} \right) \times 100
\]
3.6 Submaximal treadmill test

The submaximal treadmill assessment was designed to elicit a controlled external work rate which was increased via firstly an increase in speed (stage 1) and secondly and increase in gradient (stage 2). The treadmill speed for each participant was initially set to the pre-defined relative speeds determined during the 400m walk. Pre-exercise resting measures were collected with the participant in a seated position, followed immediately by the submaximal treadmill protocol. Heart rate and whole body oxygen consumption were measured continuously. On completion of the treadmill protocol, participants were required to recover in a standardized supine position. This assessment was also completed before and after the supplementation period.

3.6.1 Pre-exercise

The protocol began with participants seated for 5 minutes. Participants were required to breathe normally and remain quiet. Pre-exercise resting measures, including heart rate, oxygen consumption and muscle oxygen saturation were continuously measured.

3.6.2 Treadmill protocol

Following the pre-exercise resting phase, participants were moved to the treadmill to begin the submaximal walking protocol. The protocol was adapted from the Balke Treadmill Test (Balke and Ware 1959) and consisted of 2 stages. Stage 1 lasted 9 minutes and began on a 1% gradient. Adjustments were made to treadmill speed, increasing by 0.5km/h every 3 minutes, reaching a peak speed which was calculated as 70-75% of maximum 400m walking speed. Stage 2 lasted 6 minutes and during stage 2 the treadmill gradient increased by 2% every 3 minutes, reaching a final gradient of 5% during the last 3 minutes. Treadmill speed remained constant during stage 2. Heart rate, RPE, oxygen consumption and muscle oxygen saturation were continuously measured.
3.6.3  *Post treadmill recovery phase*

After completing the treadmill walk, participants were immediately moved to a plinth where they lay supine for a period of 10 minutes with room lights turned off. There were no external influences such as noise or movement and participants were asked to remain quiet and still during the 10 minute recovery phase.

3.6.4  *Experimental measures and analysis*

3.6.4.1.  *Heart rate*

Heart rate was continuously collected on a beat to beat (ventricular depolarisation) basis during the visit. Heart rate data was downloaded to the Polar Protraine 5 software program (Finland). The files were then converted to text files which allowed them to be imported into Kubios heart rate variability program (Department of Physics of the University of Kuopio, Finland) where they were analysed. Resting heart rate was calculated as the average heart rate during the last 3 minutes of the pre-exercise seated rest. Exercise heart rate for stage 1 and stage 2 was determined by averaging the final 60 seconds of each stage, respectively.

3.6.4.2.  *Heart rate recovery*

Heart rate recovery after the completion of the treadmill protocol was determined by applying a logit transformation model to the recovery heart rate values which allowed for a single phase model of half recovery times to be established (Stupnicki, Gabrys et al. 2010). This method allows comparisons between participant’s time to half recovery ($t_{1/2}$). A spreadsheet was created in Microsoft Excel (2016) which allowed instantaneous heart rate values to be plotted at pre-determined intervals during the 10 minute supine recovery phase, in addition to overnight minimum heart rate and exercising peak rate heart rate. Heart rates were plotted at every 15 second
mark for the first 3 minutes, followed by at every 30 second mark for the next 3 minutes and finally at every 60 second mark for the remaining 4 minutes of supine recovery. Following this, the net difference between the overnight resting heart rate and the instantaneous heart rates was calculated. An example of this step using trial data is shown. The net heart rate values were then converted to log transformed values. An example using trial data is shown figure 3.2. Finally log transformed data was converted to decimal logit values using the following recommended formula (89):

**Equation 2:** \( \text{logit}(x_i) = \log\left(\frac{x_i}{x_m-x_i}\right) \)

Where \( x_i \) is the net value of heart rate at time point i, and \( x_m \) is the peak heart rate.

From here a half time was established from the time to half intercept. An example using trial data is shown in figure 3.3.
Figure 3.1. Net heart rate (mean ± SEM) during post-exercise recovery.

Data taken from baseline assessments to represent the net heart rate drop after the submaximal treadmill protocol. Recovery time immediately following exercise is shown in seconds on the x-axis. Net heart rate which was calculated as peak heart rate minus minimum heart rate is shown on the y-axis in beats per minute.

Figure 3.2. Heart rate log values (mean ± SEM) during post-exercise recovery.

Data taken from baseline assessments to represent the log heart rate drop after the submaximal treadmill protocol. Recovery time immediately following exercise is shown in seconds on the x-axis. Net heart rate values were logged and they are shown on the y-axis.

Figure 3.3. Logit heart rate values (mean ± SEM) during post-exercise recovery.

The value of log time at logit = 0 corresponds to log half-recovery time ($t_{1/2}$).
3.6.5  *Muscle oxygen*

Muscle oxygen saturation was collected at 2 second intervals for the entire visit. Near-infrared spectroscopy data files were downloaded as Microsoft Excel files. Resting tissue saturation index was calculated by averaging the last 3 minutes of the pre-exercise seated rest. Exercise tissue saturation for stage 1 and stage 2 was determined by averaging the final 60 seconds of each stage, respectively. A spreadsheet was created to calculate relative tissue saturation index for each treadmill stage by using the following equation,

\[
\text{Equation 3: } \left( \frac{\text{Exercising TSI} - \text{Resting TSI}}{\text{Resting TSI}} \right) \times 100
\]

3.6.6  *Oxygen consumption*

Participants were fitted with a Hans Rudolf two-way non-rebreathing face mask (Hans Rudolf inc, Kansas, USA). Respiratory data was collected over a 15 second rolling average during seated rest and during the treadmill protocol using expired gas analysis (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Utah, USA). The system was calibrated daily using a 2-point calibration of room air (21% oxygen, 0.03% carbon dioxide) and scientifically graded gas (15.87% oxygen, 4.03% carbon dioxide). Breath by breath data sampled included oxygen consumption, carbon dioxide production, tidal volume, breathing frequency, inspiratory duration, and expiratory duration. Resting oxygen consumption was collected during the 5 minute pre-exercise seated rest and the final 3 minutes were averaged and used for analysis. A single sample of the final 60 second oxygen consumption at each treadmill stage was calculated and along with work rate, was used to determine oxygen cost of exercise. Net oxygen consumption was calculated by subtracting the
resting oxygen consumption from the gross oxygen consumption during the final 60 seconds of each treadmill stage.

Work rate was calculated using the following equation,

\[
Work \ rate \ (kg \cdot m \cdot \text{min}^{-1}) = \frac{(body \ mass \times (sine \ angle \times \ distance))}{3 \ minutes}
\]

Finally, net oxygen consumption and work rate were used to determine oxygen cost of walking.

\[
Cost \ of \ walking \ (mL \cdot m^{-1}) = \frac{Net \ oxygen \ consumption \ (mL/kg/min)}{Work \ rate \ (kg/m/min)}
\]

3.6.7 Rating of perceived exertion

The 15-point Borg Rating of Perceived Exertion (RPE) scale was used to measure participant’s subjective exercise intensity (where 6 = very, very light, 20 = very, very hard) (Borg 1982). Participants were asked “how hard are you exercising” within the last 60 seconds of each treadmill stage.

3.7 Blood sample collection and storage

Whole blood sample (plasma, white blood cells, platelets, and red blood cells) collection was performed before and after the 16 weeks of supplementation using a dried blood spot method (OmegaQuant, South Dakota, United States). This test is a minimal burden form of blood sampling which has been validated per the Guidance for Industry: Bioanalytical Method
Evaluation (FDA; May, 2001) and previously used to determine omega-3 concentrations in red blood cell membranes (Johnston, Deuster et al. 2013, Baack, Puumala et al. 2015). The finger of the participant was cleaned with an alcohol swab. A lancet containing a spring-loaded needle was used to collect the blood spot. A drop of blood was collected on filter paper that was pre-treated with an antioxidant cocktail (Fatty Acid Preservative Solution, FAPS™) and allowed to dry at room temperature for 15 minutes. Samples were stored at -80 degrees celsius and then shipped to OmegaQuant Analytics for fatty acid analysis. One hole punch of the dry blood spot was transferred to a screw-cap glass vial followed by addition of BTM (methanol containing 14% boron trifluoride, toluene, methanol; 35:30:35 v/v/v) (Sigma-Aldrich, St. Louis, MO). The vial was briefly vortexed and heated in a hot bath at 100°C for 45 minutes. After cooling, hexane (EMD Chemicals, USA) and HPLC grade water was added, the tubes were recapped, vortexed and centrifuged help to separate layers. An aliquot of the hexane layer was transferred to a GC vial. GC was carried out using a GC-2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP-2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 um film thickness; Supelco, Bellefonte, PA).

Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of RBC (GLC OQ-A, NuCheck Prep, Elysian, MN) which was also used to construct individual fatty acid calibration curves. The following 24 fatty acids (by class) were identified: saturated (14:0, 16:0, 18:0, 20:0, 22:0 24:0); cis monounsaturated (16:1, 18:1, 20:1, 24:1); trans (16:1, 18:1*, 18:2* - see below for more details); cis n-6 polyunsaturated (18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5); cis n-3 polyunsaturated (18:3, 20:5, 22:5, 22:6). Fatty acid composition was expressed as a percent of total identified fatty acids. The omega-3 index is defined as the sum of 20:5n-3 (EPA) and 22:6n-
3 (DHA) adjusted by a regression equation \((r = 0.97)\) to predict the omega-3 index in the RBC (Johnston, Deuster et al. 2013).

*The chromatographic conditions used in this study were sufficient to isolate the C16:1\textit{trans} isomers and the C18:2 \(\Delta 9\text{-}12c, 9\text{-}12t,\) and \(9c\text{-}12t\) isomers; the latter is reported as C18:2n6t. However, each individual C18:1 \textit{trans} molecular species (i.e., C18:1 \(\Delta 6\) thru \(\Delta 13\)) could not be separated but appeared as 2 blended peaks that eluted just before oleic acid. The areas of these 2 peaks were summed and referred to a C18:1 \textit{trans}. 

4. Ambulatory cardiac monitoring

4.1 Overview

Ambulatory cardiac monitoring was carried out before and after the supplementation period. Participants were fitted with a wearable cardiac monitor (eMotion Faros 180) which is a low burden device that collects ECG quality heart recordings (Mega Electronics Ltd, Finland). The device consists of a small sensor and 3 cables with disposable electrode patches (Ambu BlueSensor VLC Electrodes, Ballerup Denmark). Participants were required to wear the monitor for 24 hours in order to capture overnight sleep on at least 1 night.

4.2 Protocol

Participants were fitted with the cardiac monitor prior to leaving the laboratory in accordance with manufacturer recommendations. The first electrode (right arm) which contained the portable monitor was placed in the 2nd intercostal space medial to the right deltoid. The second electrode (left arm) was placed in the 2nd intercostal space medial to the left deltoid. The third electrode (left leg) was placed between the left pectoral muscle and the iliac crest. Participants were asked to remove the monitor if it may come in contact with water (e.g. swimming, showering), and then return the monitor and electrodes to the same position once finished. Additional electrode patches were provided to participants in order to reattach the device after water based activities.

4.3 Analysis

ECG and activity data from the monitor were downloaded as EDF files to the desktop. The downloaded files were then imported into HRV-Scanner Software (Biosign, Ottenhofen Germany). The files were placed through an automatic filter which is part of the HRV-Scanner program. Filtering removed artefacts and noise from the signal. Sleep was determined when the
accelerometer which is built in to the device recorded an extended supine period. The trace was visually inspected for when heart rate became steady and analysis started from the beginning of that point. Overnight R-R times were collected and heart rate and heart rate variability was calculated over both the entire night as well a 10 minute period during the night where heart rate was at its lowest. The lowest 10 minute period was determined by visual inspection of the trace. Time, nonlinear, and frequency parameters were analysed for heart rate variability. In addition, the ratio between overnight heart rate and awake resting heart rate was calculated to assess the degree of nocturnal dip that occurs.

### 4.3.1 *Time domain heart rate variability*

Time domain parameters measured during the entire sleep period as well as during the lowest 10 minute period during the night were the standard deviation of NN intervals (SDNN), and the root mean square of the successive differences (RMSSD) of NN intervals. The SDNN reflects long-term cyclic components responsible for variability in the recording period. The RMSSD represents short term dynamic changes in beat to beat variability and is a good indicator of sympathovagal balance (Otzenberger, Gronfier et al. 1998).

### 4.3.2 *Nonlinear domain heart rate variability*

A Poincaré plot was used for quantitative-visual analysis of beat-to-beat variability. The Poincaré plot is a scattergram of the current RR interval plotted as a function of the previous one. An ellipse is then fitted along the line of identity to the plot and is used to determine SD1 and SD2 (figure 4.1). Dispersion of the points perpendicular to the line of identity are represented by SD1 which is an index of instantaneous beat-to-beat variability (parasympathetic) (Kamen, Krum et al. 1996). The points along the line of identity are represented by SD2. The length of the SD2 along the line
of identity reflects continuous beat-to-beat variability (sympathetic) (Brennan, Palaniswami et al. 2002). The ratio of SD1:SD2 is considered to represent sympathovagal balance.

4.3.3 Frequency domain heart rate variability

The heart rate variability signal from the time series analysis was transformed with the Fourier transformation to allow spectral analysis. The transformed data was then able to be analysed on a frequency scale. High-frequency spectrum included frequencies between 0.15 and 0.40Hz. Low-frequency spectrum included frequencies between 0.04 and 0.15Hz. Very-low-frequency included frequencies of 0.04Hz and lower. The high-frequency band reflects parasympathetic activity, whereas the low-frequency band predominantly reflects sympathetic activity (Shaffer, McCraty et al. 2014).
**Figure 4.1.** Example of a Poincaré plot generated from baseline data

*RR interval is shown on the x-axis (RR\(_n\)). The subsequent RR interval is plotted on the y-axis (RR\(_{n+1}\)). SD1 represents dispersion of points perpendicular to the line of identity and indicates parasympathetic activity (Kamen, Krum et al. 1996). SD2 represents point along the line of identity and indicates sympathetic activity (Brennan, Palaniswami et al. 2002).*
4.4 Experimental standardisation

All experimental laboratory trials were conducted in the University of Wollongong’s exercise physiology patient simulation laboratory (room temperature 22°C, 35% relative humidity). Assessments requiring an additional room were conducted at the UniActive Sports Centre (University of Wollongong), which is a temperature-controlled room. Participants were instructed to consume 10 millilitres of water per 1kg body weight prior to testing to maintain hydration status. Testing equipment used during the visits was prepared and calibrated prior to participant arrival.

4.5 Statistical analysis

Using a power of 0.8, an estimated average standard deviation of 3, and the ability to detect a 4 beat per minute difference in resting heart rate, a minimum number of 8 participants per group was calculated. To allow for a 20% drop-out rate, 10 participants per supplement group were recruited for this study. Analysis of the collected data was conducted using the Statistix 10 for Windows (Analytical Software, Tallahassee FL, USA) and Graphpad (Prism 5, La Jolla, CA, USA) software packages. For association relationships, a linear regression model was applied to the relevant variables. For outcome measures directly pertaining to the primary aims of the study, a repeated measures analysis of variance (ANOVA) was used, with diet supplement (control, fish oil groups) and time (baseline and 16-week post treatment condition) main effects, and supplement * time interaction. In addition, mean differences between pre- and post-supplementation for the control group compared to the mean differences pre- and post-supplementation for the DHA-rich fish oil group were analysed by one way ANOVA. Where significant difference was established, a post hoc Tukey or Bonferroni analysis was conducted for comparisons of individual means. The data was expressed as mean (standard deviation) for participant characteristics or mean (standard error) for outcome measures. Alpha was set at $P<0.05$. 

45
5. Results

5.1 Demographics and functional capacity

Twenty two participants were tested at baseline and their data was used to explore age related physical function and physiology. This cross-section cohort represented healthy and physically active older adults. Not only were they free from chronic disease, including major cardiovascular and respiratory dysfunction, they were also of high physical function and walking speed. Their baseline data is presented in table 5.1.
Table 5.1. Anthropometric and physiology data at baseline for the entire cohort.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>9</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>Age (y)</td>
<td>71 (2)</td>
<td>70 (2)</td>
<td>70 (1)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.70 (0.02)</td>
<td>1.59 (0.01)</td>
<td>1.63 (0.02)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.39 (2.63)</td>
<td>58.05 (2.29)</td>
<td>65.96 (2.67)</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.98 (1.13)</td>
<td>22.98 (1.01)</td>
<td>24.62 (0.85)</td>
</tr>
<tr>
<td>MNA®-SF score</td>
<td>13.63 (0.14)</td>
<td>13.46 (0.66)</td>
<td>13.52 (0.60)</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>134 (4)</td>
<td>131 (4)</td>
<td>132 (3)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76 (2)</td>
<td>74 (2)</td>
<td>75 (2)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>66 (3)</td>
<td>68 (2)</td>
<td>67 (2)</td>
</tr>
<tr>
<td><strong>Physical Function</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left grip (kg)</td>
<td>32.89 (2.79)</td>
<td>17.77 (1.24)</td>
<td>23.96 (2.09)</td>
</tr>
<tr>
<td>Right grip (kg)</td>
<td>34.78 (2.57)</td>
<td>17.81 (1.41)</td>
<td>24.75 (2.24)</td>
</tr>
<tr>
<td>Left pinch (kg)</td>
<td>8.94 (0.61)</td>
<td>5.42 (0.37)</td>
<td>6.86 (0.50)</td>
</tr>
<tr>
<td>Right pinch (kg)</td>
<td>9.22 (0.71)</td>
<td>5.73 (0.36)</td>
<td>7.16 (0.51)</td>
</tr>
<tr>
<td>Sit to stand (reps)</td>
<td>21.56 (2.33)</td>
<td>17.15 (0.93)</td>
<td>18.96 (1.17)</td>
</tr>
<tr>
<td>Time up and go (s)</td>
<td>4.13 (0.23)</td>
<td>4.46 (0.22)</td>
<td>4.33 (0.16)</td>
</tr>
<tr>
<td>Bicep Curl (reps)</td>
<td>22.33 (1.75)</td>
<td>17.46 (1.14)</td>
<td>19.46 (1.09)</td>
</tr>
<tr>
<td><strong>Walking Speed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20m usual speed (m/s)</td>
<td>1.28 (0.02)</td>
<td>1.24 (0.05)</td>
<td>1.26 (0.03)</td>
</tr>
<tr>
<td>20m rapid speed (m/s)</td>
<td>1.89 (0.09)</td>
<td>1.73 (0.06)</td>
<td>1.79 (0.05)</td>
</tr>
<tr>
<td>400m speed (m/s)</td>
<td>1.64 (0.06)</td>
<td>1.49 (0.05)</td>
<td>1.55 (0.04)</td>
</tr>
</tbody>
</table>

Cardiovascular parameters measured supine at rest; Sit to stand = no. of reps in 30s; Timed up and go = stand – 4m walk – sit; Bicep curl = lifting 2kg (female), 3kg (male) No. reps in 30s; 400m speed = average speed over 400m walking. Values are mean (SEM). Abbreviations: BMI, body mass index; MNA®-SF, Mini-Nutritional Assessment – Short Form; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Data from the International Physical Activity Questionnaire are presented in table 5.2. Participants achieved an average of 79 minutes of moderate intensity physical activity per day which far exceeded the recommended 30 minutes of moderate intensity physical activity on most, preferably all, days for older adults (Australian Government 2013).

Table 5.2. International Physical Activity Questionnaire data for males (n=9) and females (n=13) at baseline for the entire cohort (n=22).

<table>
<thead>
<tr>
<th>IPAQ</th>
<th>Male</th>
<th>Female</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walking min</td>
<td>542 (119)</td>
<td>851 (204)</td>
<td>725 (146)</td>
</tr>
<tr>
<td>Walk MET-min</td>
<td>1789 (656)</td>
<td>2810 (673)</td>
<td>2392 (482)</td>
</tr>
<tr>
<td>Moderate activity min</td>
<td>640 (324)</td>
<td>493 (95)</td>
<td>553 (140)</td>
</tr>
<tr>
<td>Moderate MET-min</td>
<td>2562 (1295)</td>
<td>1975 (379)</td>
<td>2215 (560)</td>
</tr>
<tr>
<td>Vigorous activity min</td>
<td>246 (65)</td>
<td>108 (59)</td>
<td>164 (45)</td>
</tr>
<tr>
<td>Vigorous MET-min</td>
<td>1968 (523)</td>
<td>867 (470)</td>
<td>1318 (362)</td>
</tr>
<tr>
<td>Total MET-min</td>
<td>6320 (1597)</td>
<td>5653 (1163)</td>
<td>5926 (927)</td>
</tr>
</tbody>
</table>

*Self-reported physical activity data over 7 days. Values are mean (SEM). Abbreviations: MET, metabolic equivalent of task.*
5.2 The oxygen cost of walking in the older adult

The relationship between self-selected walking speed collected during the 20m and 400m walk tests and anthropometric measures, in addition to self-reported physical activity and the oxygen cost of walking collected during stage 1 and stage 2 of the treadmill protocol was assessed using linear regression. Height was positively associated with 20m rapid and 400m rapid walking speeds, but not usual walking speed (table 5.3). Age and BMI was not significantly associated with any walking speed.

There was a significant inverse relationship between usual and rapid walking speed over 20m and 400m and the oxygen cost of walking at stage 1 (increasing speed) and stage 2 (increasing gradient) of the treadmill protocol, whereby participants with lower oxygen cost of walking achieved faster walking speeds (table 5.4, figure 5.1).
Table 5.3. Association between walking speed and age, height and BMI for the entire cohort (n=22).

<table>
<thead>
<tr>
<th>Walking Speed</th>
<th>Age (y)</th>
<th>Height (m)</th>
<th>BMI (kg/m²)</th>
<th>R²</th>
<th>R²</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>20m usual speed (m/s)</td>
<td>0.14</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20m rapid speed (m/s)</td>
<td>0.14</td>
<td>0.28*</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400m rapid speed (m/s)</td>
<td>0.17</td>
<td>0.31**</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01. Abbreviations: BMI, body mass index.

Table 5.4. Association between walking speed and treadmill oxygen cost of walking.

<table>
<thead>
<tr>
<th>Walking speed</th>
<th>Treadmill oxygen cost of walking (mL/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treadmill Stage 1</td>
</tr>
<tr>
<td></td>
<td>R²</td>
</tr>
<tr>
<td>20m usual speed (m/s)</td>
<td>0.37**</td>
</tr>
<tr>
<td>20m rapid speed (m/s)</td>
<td>0.35**</td>
</tr>
<tr>
<td>400m rapid speed (m/s)</td>
<td>0.26*</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01. Treadmill stage 1 (n=19), treadmill stage 2 (n=15).
Figure 5.1. Linear regression of 20m usual walking speed and oxygen cost of walking at (A) stage one (A) and stage two (B) of treadmill walking, 20m rapid walking speed and oxygen cost of walking at stage one (C) and stage two (D) of treadmill walking, and 400m rapid walking speed and oxygen cost of walking at stage one (E) and stage two (F) of treadmill walking.

Treadmill stage one (n=19), treadmill stage two (n=15).
Linear regression of the oxygen cost of walking and the absolute heart rate during the treadmill protocol at stage 1 and stage 2 are presented in table 5.5. The oxygen cost of walking was significantly associated with heart rate during both stages of the treadmill protocol, whereby the greater the oxygen cost of walking, the greater the heart rate had to be to provide oxygen (table 5.5, figure 5.2).

**Table 5.5.** Linear regression of heart rate and oxygen cost of walking during the treadmill protocol.

<table>
<thead>
<tr>
<th>Heart rate (bpm)</th>
<th>Treadmill Stage 1</th>
<th>Treadmill Stage 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( R^2 )</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>Treadmill Stage 1</td>
<td>0.44**</td>
<td></td>
</tr>
<tr>
<td>Treadmill Stage 2</td>
<td></td>
<td>0.57**</td>
</tr>
</tbody>
</table>

**P<0.01. Treadmill stage 1 (n=19), treadmill stage 2 (n=15).**

**Figure 5.2.** Linear regression of heart rate and oxygen cost of walking at stage one (A) and stage two (B) of the treadmill protocol.

*Treadmill stage one (n=19), treadmill stage two (n=15).*
Data from self-reported physical activity and fatigue collected from the International Physical Activity Questionnaire – Elderly and the Pittsburg Fatigability Scale and the oxygen cost of walking during the submaximal treadmill protocol are presented in table 5.6. There were no significant relationships for either self-reported physical activity or fatigue and oxygen cost of walking during the treadmill protocol.

Table 5.6. Linear regression of treadmill oxygen cost of walking and self-reported physical activity and fatigue.

<table>
<thead>
<tr>
<th></th>
<th>Treadmill oxygen cost of walking (mL/m)</th>
<th>Treadmill Stage 1</th>
<th>Treadmill Stage 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R²</td>
<td>R²</td>
</tr>
<tr>
<td><strong>IPAQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walk MET-min</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Moderate MET-min</td>
<td></td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Vigorous MET-min</td>
<td></td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Total MET-min</td>
<td></td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>PFS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical fatigue</td>
<td></td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>Mental fatigue</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Treadmill stage 1 and IPAQ (n=19), treadmill stage 2 and IPAQ (n=16). Treadmill stage 1 and PFS (n=17), treadmill stage 2 and PFS (n=14). Abbreviations: IPAQ, International Physical Activity Questionnaire; MET, metabolic equivalents of task; PFS, Pittsburgh Fatigability Scale.
5.3 Agreement between self-reported nutrition data and blood fatty acids

To determine whether self-reported nutrition data reflected red blood cell fatty acids, a linear regression model was used for the PUFA FFQ and for the 3-day food record and corresponding red blood cell fatty acids at baseline. There was no relationship at baseline between red blood cell markers and self-reported LC n-3 PUFA from the PUFA FFQ (table 5.7) or the 3-day food record (table 5.8).

Table 5.7. Relationship between whole blood fatty acids and red blood cell equivalent omega-3 index and self-reported fatty acids from the PUFA food frequency questionnaire.

<table>
<thead>
<tr>
<th></th>
<th>Whole blood fatty acids</th>
<th>RBC EPA+DHA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA</td>
<td>DHA</td>
<td>EPA+DHA</td>
</tr>
<tr>
<td>PUFA FFQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHA (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA+DHA (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA+DHA (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=15. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; FFQ, food frequency questionnaire

To determine whether there was a relationship between the two self-reported collection tools and their reporting of long chain fatty acids, the PUFA food frequency questionnaire and the 3-day food record were assessed for agreement. There was no significant relationship between these two measures (table 5.9).
Table 5.8. Relationship between whole blood fatty acids and red blood cell equivalent omega-3 index and self-reported fatty acids from the 3-day food record.

<table>
<thead>
<tr>
<th></th>
<th>Whole blood fatty acids</th>
<th>RBC EPA+DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA R²</td>
<td>DHA R²</td>
</tr>
<tr>
<td>3-day food record</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA (g/d)</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>DHA (g/d)</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>EPA+DHA (g/d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA+DHA (g/d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=14. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 5.9. Relationship between self-reported fatty acids from the 3-day food record and PUFA food frequency questionnaire.

<table>
<thead>
<tr>
<th></th>
<th>PUFA FFQ (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA R²</td>
</tr>
<tr>
<td>3D-Food Record</td>
<td></td>
</tr>
<tr>
<td>EPA (g/d)</td>
<td>0.01</td>
</tr>
<tr>
<td>DHA (g/d)</td>
<td></td>
</tr>
<tr>
<td>EPA+DHA (g/d)</td>
<td></td>
</tr>
</tbody>
</table>

N=14. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid; FFQ, food frequency questionnaire.
5.4 Agreement between physical function and blood fatty acids

There was a significant relationship between right hand pinch strength and whole blood DHA (P<0.05) (table 5.10). Left hand pinch strength was significantly associated with DHA, EPA+DHA, and the omega-3 index (P<0.05) (table 5.10). No other significant relationships were identified between the long chain omega-3 fatty acid and walking capacity.

Table 5.10. Relationship between whole blood fatty acids and red blood cell equivalent omega-3 index at baseline and heart rate, oxygen cost of treadmill walking, walking speed, and grip strength.

<table>
<thead>
<tr>
<th></th>
<th>Whole blood fatty acids</th>
<th>RBC EPA+DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>R²</td>
</tr>
<tr>
<td>HR stage 1 treadmill walking (bpm)</td>
<td>0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>HR stage 2 treadmill walking (bpm)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Oxygen cost of treadmill walking stage 1 (mL/m)</td>
<td>0.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Oxygen cost of treadmill walking stage 2 (mL/m)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>20m usual speed (s)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>20m rapid speed (s)</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>400m rapid speed (s)</td>
<td>0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Right hand grip strength (kg)</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>Left hand grip strength (kg)</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>Right hand pinch strength (kg)</td>
<td>0.01</td>
<td>0.19*</td>
</tr>
<tr>
<td>Left hand pinch strength (kg)</td>
<td>0.03</td>
<td>0.27*</td>
</tr>
</tbody>
</table>

*P<0.05. Subject numbers are reported in the table for each variable. Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HR, heart rate.
5.5 Fish oil supplementation and the effects on physiological and physical function

Of the 22 participants tested at baseline, 21 met inclusion into the study. One participant had a high self-reported fish consumption and was excluded. Of the 21 participants that met inclusion, 2 participants chose not to continue following the baseline assessments. Therefore, 19 participants were allocated to a supplement group. By 16 weeks, 1 participant was excluded due to non-compliance (self-reported increase in a fish oil supplement) and 1 participant had a baseline omega-3 index >8% which was confirmed via blood sample analysis. This resulted in 17 participants, who met all the criteria, successfully completing the 16 week trial (89% completion rate). This included 8 participants for the control group and 9 participants for the fish oil group. Anthropometric and demographic data are presented in table 5.11. The study recruitment and participant completion is shown in figure 2.2.

Table 5.11. Anthropometric and demographic data, before and after supplementation.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Fish Oil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Anthropometric</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>70 (1)</td>
<td></td>
<td>70 (1)</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.62 (0.02)</td>
<td>1.62 (0.02)</td>
<td>1.65 (0.03)</td>
<td>1.65 (0.03)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.75 (4.88)</td>
<td>66.43 (4.86)</td>
<td>67.03 (4.13)</td>
<td>66.43 (4.00)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.95 (1.57)</td>
<td>25.21 (1.55)</td>
<td>24.78 (1.44)</td>
<td>24.59 (1.47)</td>
</tr>
</tbody>
</table>

*Control (n=8), fish oil (n=9). Values are mean (SEM). Abbreviations: BMI, body mass index.*
5.6 Diet assessment

Data from the 3-day food record are presented in table 5.12. All participants met the estimated energy requirements (EER) for their age, body mass index and activity level (National Health and Medical Research Council 2006). Participants exceeded the recommended dietary intake (RDI) for protein in older adults based on weight which is 1.07g/kg for males (~80g/d), and 0.94g/kg for females (~56g/d). In addition, participants also exceeded the protein intake that is advised for physically active older adults of 1.2g/kg (Bauer, Biolo et al. 2013), however they were still within the suggested dietary target of no more than 25% of protein as energy (National Health and Medical Research Council 2006). Participants consumed approximately 1.6g/kg of protein in the current study. There were no significant differences in any nutrient at baseline or after supplementation (P>0.05).
Table 5.12. Nutrition data collected from the 3-day food record for control (n=7) and fish oil (n=7) groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fish Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>8967 (802)</td>
<td>8977 (488)</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>106 (9)</td>
<td>105 (9)</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>88 (11)</td>
<td>82 (6.9)</td>
</tr>
<tr>
<td>Saturated fat (g/d)</td>
<td>30 (3)</td>
<td>25 (3)</td>
</tr>
<tr>
<td>Trans fat (g/d)</td>
<td>1.3 (0.2)</td>
<td>1.1 (0.2)</td>
</tr>
<tr>
<td>Polyunsaturated fat (g/d)</td>
<td>17.0 (4.2)</td>
<td>14.7 (2.6)</td>
</tr>
<tr>
<td>Linoleic acid (g/d)</td>
<td>13.5 (3.5)</td>
<td>11.8 (1.5)</td>
</tr>
<tr>
<td>a-linoleic acid (g/d)</td>
<td>2.9 (0.8)</td>
<td>1.7 (0.6)</td>
</tr>
<tr>
<td>EPA (g/d)</td>
<td>0.2 (0.1)</td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td>DPA (g/d)</td>
<td>0.1 (0.0)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>DHA (g/d)</td>
<td>0.2 (0.1)</td>
<td>0.4 (0.2)</td>
</tr>
<tr>
<td>Monounsaturated fat (g/d)</td>
<td>34 (4)</td>
<td>36 (3)</td>
</tr>
<tr>
<td>Cholesterol (mg/d)</td>
<td>229 (31)</td>
<td>239 (58)</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>205 (17)</td>
<td>226 (19)</td>
</tr>
<tr>
<td>Sugar (g/d)</td>
<td>109 (12)</td>
<td>103 (9)</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>40 (7)</td>
<td>33 (3)</td>
</tr>
<tr>
<td>% Energy (kJ) from Protein</td>
<td>20.2 (1.1)</td>
<td>19.9 (1.3)</td>
</tr>
<tr>
<td>% Energy (kJ) from Fat</td>
<td>35.9 (1.3)</td>
<td>33.6 (2.3)</td>
</tr>
<tr>
<td>% Energy (kJ) from Carbohydrate</td>
<td>37.7 (1.4)</td>
<td>41.6 (2.5)</td>
</tr>
<tr>
<td>Ash (g/d)</td>
<td>20.2 (1.6)</td>
<td>17.2 (1.7)</td>
</tr>
<tr>
<td>Thiamin (mg/d)</td>
<td>2.0 (0.2)</td>
<td>1.9 (0.4)</td>
</tr>
<tr>
<td>Riboflavin (mg/d)</td>
<td>3.0 (0.2)</td>
<td>2.0 (0.2)</td>
</tr>
<tr>
<td>Niacin (mg/d)</td>
<td>25.9 (3.3)</td>
<td>25.7 (3.6)</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>211 (601)</td>
<td>137 (38)</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>16.3 (3.2)</td>
<td>13.1 (2.5)</td>
</tr>
<tr>
<td>Vitamin B6 (mg/d)</td>
<td>2.0 (0.3)</td>
<td>2.0 (0.2)</td>
</tr>
<tr>
<td>Vitamin B12 (µg/d)</td>
<td>5.6 (0.6)</td>
<td>4.5 (0.6)</td>
</tr>
<tr>
<td>Total Folate (µg/d)</td>
<td>960 (374)</td>
<td>465 (32)</td>
</tr>
<tr>
<td>Folic acid (µg/d)</td>
<td>143 (33)</td>
<td>148 (33)</td>
</tr>
<tr>
<td>Total Vitamin A (µg/d)</td>
<td>1294 (203)</td>
<td>986 (302)</td>
</tr>
<tr>
<td>Sodium (mg/d)</td>
<td>1930 (154)</td>
<td>2025 (409)</td>
</tr>
<tr>
<td>Potassium (mg/d)</td>
<td>4444 (506)</td>
<td>3642 (221)</td>
</tr>
<tr>
<td>Magnesium (mg/d)</td>
<td>506 (64)</td>
<td>415 (25)</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>1312 (108)</td>
<td>813 (92)</td>
</tr>
<tr>
<td>Phosphorus (mg/d)</td>
<td>1932 (157)</td>
<td>1670 (147)</td>
</tr>
<tr>
<td>Iron (mg/d)</td>
<td>14.5 (1.6)</td>
<td>13.2 (1.1)</td>
</tr>
<tr>
<td>Zinc (mg/d)</td>
<td>11.9 (0.9)</td>
<td>10.9 (0.7)</td>
</tr>
</tbody>
</table>

Values are mean (SEM). Abbreviations: EPA, eicosapentaenoic acid; DPA, Docosapentaenoic acid; DHA, docosahexaenoic acid.
5.7 Fatty acids

Fatty acid analysis began at myristic acid (14:0) and continued to docosahexaenoic acid (22:6n-3). Finally, the omega-3 index was defined as the sum of 20:5n-3 (EPA) and 22:6n-3 (DHA) adjusted by a regression equation (r = 0.97) to predict the omega-3 index in the RBC. Omega-3 fatty acid composition was expressed as a percent of total identified fatty acids (table 5.13). All fatty acids are shown in table 5.13.

There were no significant differences between groups at baseline for any fatty acid (P>0.05) (table 5.13). There was an increase in EPA (20:5n-3) (P<0.05), DHA (22:6n-3) (P<0.01), and the omega-3 index (P<0.01) within the fish oil group post supplementation (table 5.13, figure 5.3). There was a difference in DHA (22:6n-3) (P<0.01), DPA (20:2n-6) (P<0.05) and the omega-3 index (P<0.01) between control and fish oil post supplement (table 5.13, figure 5.3). Finally, there was a decrease in eicosadienoic acid (20:2n-6) within the fish oil group (P<0.05) (table 5.13).
Table 5.13. Fatty acid composition expressed as a percent of total identified fatty acids from a whole blood, dry sample.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Control</th>
<th></th>
<th></th>
<th>Fish Oil</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>14:0</td>
<td>1.09 (0.20)</td>
<td>1.21 (0.18)</td>
<td>1.04 (0.20)</td>
<td>0.83 (0.11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>23.80 (0.76)</td>
<td>23.04 (0.77)</td>
<td>22.29 (0.85)</td>
<td>21.81 (0.67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1n-7t</td>
<td>0.20 (0.01)</td>
<td>0.18 (0.01)</td>
<td>0.14 (0.02)</td>
<td>0.17 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1n-7</td>
<td>1.48 (0.23)</td>
<td>1.28 (0.22)</td>
<td>1.45 (0.28)</td>
<td>1.03 (0.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>11.11 (0.29)</td>
<td>11.86 (0.31)</td>
<td>11.64 (0.38)</td>
<td>11.73 (0.36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1t</td>
<td>0.61 (0.05)</td>
<td>0.62 (0.05)</td>
<td>0.48 (0.05)</td>
<td>0.43 (0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:1n-9</td>
<td>22.92 (0.80)</td>
<td>21.97 (0.85)</td>
<td>21.15 (1.06)</td>
<td>21.81 (0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2n-6t</td>
<td>0.24 (0.02)</td>
<td>0.30 (0.02)</td>
<td>0.24 (0.03)</td>
<td>0.22 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2n-6</td>
<td>18.65 (1.21)</td>
<td>18.76 (1.41)</td>
<td>20.83 (1.68)</td>
<td>20.32 (1.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td>0.21 (0.01)</td>
<td>0.23 (0.02)</td>
<td>0.24 (0.02)</td>
<td>0.24 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:3n-6</td>
<td>0.32 (0.04)</td>
<td>0.30 (0.04)</td>
<td>0.25 (0.04)</td>
<td>0.17 (0.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:1n-9</td>
<td>0.31 (0.02)</td>
<td>0.33 (0.04)</td>
<td>0.39 (0.04)</td>
<td>0.30 (0.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.43 (0.04)</td>
<td>0.43 (0.03)</td>
<td>0.65 (0.19)</td>
<td>0.50 (0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:2n-6</td>
<td>0.21 (0.01)</td>
<td>0.21 (0.01)</td>
<td>0.26 (0.02)</td>
<td>0.20 (0.01)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:0</td>
<td>0.45 (0.05)</td>
<td>0.51 (0.05)</td>
<td>0.42 (0.04)</td>
<td>0.51 (0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:3n-6</td>
<td>1.48 (0.09)</td>
<td>1.50 (0.10)</td>
<td>1.52 (0.09)</td>
<td>1.36 (0.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>8.70 (0.62)</td>
<td>9.04 (0.63)</td>
<td>9.13 (0.53)</td>
<td>8.61 (0.47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24:0</td>
<td>0.61 (0.12)</td>
<td>0.77 (0.08)</td>
<td>0.58 (0.10)</td>
<td>0.74 (0.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5n-3</td>
<td>1.04 (0.14)</td>
<td>0.93 (0.05)</td>
<td>0.96 (0.09)</td>
<td>1.38 (0.12)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24:1n-9</td>
<td>0.65 (0.14)</td>
<td>0.81 (0.09)</td>
<td>0.66 (0.12)</td>
<td>0.79 (0.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:4n-6</td>
<td>0.90 (0.09)</td>
<td>1.07 (0.10)</td>
<td>1.01 (0.08)</td>
<td>0.78 (0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-6</td>
<td>0.16 (0.02)</td>
<td>0.18 (0.02)</td>
<td>0.18 (0.02)</td>
<td>0.18 (0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.31 (0.05)</td>
<td>1.37 (0.05)</td>
<td>1.34 (0.06)</td>
<td>1.15 (0.05)#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6n-3</td>
<td>3.12 (0.19)</td>
<td>3.08 (0.16)</td>
<td>3.16 (0.17)</td>
<td>4.75 (0.22)**†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O3I</td>
<td>6.06 (0.29)</td>
<td>5.88 (0.18)</td>
<td>6.01 (0.23)</td>
<td>8.31 (0.37)**†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Omega-3 index is equivalent to red blood cell EPA+DHA. *P<0.05 within groups, **P<0.01 within groups, †P<0.01 between groups, #P<0.05 between groups. Control (n=8), fish oil (n=9). Values are mean (SEM). Abbreviations: O3I, omega-3 index.
Figure 5.3. Whole blood EPA, DHA, and red blood cell equivalent omega-3 index for control (n=8) and fish oil (n=9) groups, pre and post supplementation.

Fatty acid values expressed as a percentage of total identified fatty acids. *P<0.05, **P<0.01.

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
The dose of EPA and DHA administered in the fish oil group was adjusted per unit of body mass and then compared to changes in red blood cell EPA, DHA, EPA+DHA, and finally the omega-3 index. There was no significant relationship between adjusted EPA and DHA dose and any red blood cell long-chain fatty acid (table 5.14).

**Table 5.14.** Bodyweight-adjusted capsule dose compared to change in long-chain fatty acids in whole blood and omega-3 index in red blood cells.

<table>
<thead>
<tr>
<th>Adjusted intake</th>
<th>Whole blood fatty acid change</th>
<th>RBC EPA+DHA change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA (mg/kg)</td>
<td>DHA (mg/kg)</td>
</tr>
<tr>
<td>EPA (mg/kg)</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>DHA (mg/kg)</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>EPA+DHA (mg/kg)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>EPA+DHA (mg/kg)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Data from fish oil group (n=9). Values are reported as $R^2$.**
5.8 Senior Fitness Assessment

Physical function data are shown in table 5.15. No significant differences were observed between the groups at baseline or after supplementation for anthropometrics or physical function, including walking speed tests (P>0.05).

Using normative self-paced walking speed data for age and gender (Bohannon and Williams Andrews 2011), participants were ranked based on whether they met the normal speed at baseline for their 20m usual walking speed assessment. There were 5 participants who did not meet the normative walking speed. Omega-3 index and overnight mean heart rate were then assessed for differences in those who did and did not meet the normative walking speed. There were no significant differences in omega-3 index or heart rate in those who did and did not meet normative walking speeds (table 5.16).
Table 5.15. Physiological and senior fitness assessment data, before and after supplementation.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fish Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129 (5)</td>
<td>128 (4)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74 (3)</td>
<td>79 (3)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>71 (3)</td>
<td>73 (4)</td>
</tr>
<tr>
<td><strong>Physical Function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left grip (kg)</td>
<td>23.63 (3.66)</td>
<td>23.53 (2.94)</td>
</tr>
<tr>
<td>Right grip (kg)</td>
<td>23.25 (3.89)</td>
<td>22.78 (3.04)</td>
</tr>
<tr>
<td>Left pinch (kg)</td>
<td>6.69 (0.83)</td>
<td>7.25 (0.94)</td>
</tr>
<tr>
<td>Right pinch (kg)</td>
<td>7.25 (0.90)</td>
<td>8.06 (1.33)</td>
</tr>
<tr>
<td>Sit to stand (reps)</td>
<td>18.75 (2.12)</td>
<td>20.13 (1.75)</td>
</tr>
<tr>
<td>Timed up and go (s)</td>
<td>4.32 (0.27)</td>
<td>4.43 (0.34)</td>
</tr>
<tr>
<td>Bicep Curl (reps)</td>
<td>19.63 (1.87)</td>
<td>22.50 (2.80)</td>
</tr>
<tr>
<td><strong>Walking Speed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20m usual speed (m/s)</td>
<td>1.27 (0.05)</td>
<td>1.34 (0.07)</td>
</tr>
<tr>
<td>20m rapid speed (m/s)</td>
<td>1.76 (0.09)</td>
<td>1.80 (0.09)</td>
</tr>
<tr>
<td>400m speed (m/s)</td>
<td>1.55 (0.06)</td>
<td>1.58 (0.07)</td>
</tr>
</tbody>
</table>

*Control (n=8), fish oil (n=9). Values are mean (SEM). Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; reps, repetitions.*

Table 5.16. Omega-3 index and overnight heart rate in those that did (n=5) and did not meet (n=12) normative walking speed for age and gender.

<table>
<thead>
<tr>
<th>Meet normative walk criteria?</th>
<th>Omega-3 index (%)</th>
<th>Mean overnight heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>6.1 (0.01)</td>
<td>61 (2)</td>
</tr>
<tr>
<td>No</td>
<td>5.7 (0.04)</td>
<td>62 (3)</td>
</tr>
</tbody>
</table>

*Values are mean (SEM). Abbreviations: bpm, beats per minute.*
Physical activity data collected from the International Physical Activity Questionnaire (IPAQ) and self-perceived physical and mental fatigue data collected from the Pittsburgh Fatigability Scale (PFS) are shown in table 5.17. There were no significant differences in reported physical activity, or reported physical or mental fatigue between groups at baseline or post-supplementation (P>0.05).

The 400m walk test was broken down into the 10 individual laps and finally a mean for all 10 laps. There was no difference in walking speed or absolute heart rate (figure 5.4). The resting tissue saturation index (%) was not different at baseline between the groups (control: pre, 71.62±6.55; fish oil: pre, 73.44±3.93), neither was the mean tissue saturation index (%) during the 400m walk (control: 43.14±1.67; fish oil: 40.82±1.71). When the tissue saturation index was expressed as a relative change there remained no difference (figure 5.4).

Table 5.17. Self-reported physical activity (IPAQ) and physical and mental fatigue (PFS).

<table>
<thead>
<tr>
<th></th>
<th>Control Pre</th>
<th>Control Post</th>
<th>Fish Oil Pre</th>
<th>Fish Oil Post</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IPAQ</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walk MET-min</td>
<td>1234 (302)</td>
<td>1070 (260)</td>
<td>1238 (149)</td>
<td>965 (270)</td>
</tr>
<tr>
<td>Moderate MET-min</td>
<td>937 (247)</td>
<td>1156 (250)</td>
<td>1073 (336)</td>
<td>1400 (449)</td>
</tr>
<tr>
<td>Vigorous MET-min</td>
<td>1406 (616)</td>
<td>1149 (504)</td>
<td>1040 (570)</td>
<td>1040 (423)</td>
</tr>
<tr>
<td>Total MET-min</td>
<td>3577 (639)</td>
<td>3375 (575)</td>
<td>3351 (728)</td>
<td>3405 (557)</td>
</tr>
<tr>
<td>Sit min</td>
<td>295 (36)</td>
<td>246 (63)</td>
<td>343 (81)</td>
<td>312 (68)</td>
</tr>
<tr>
<td><strong>PFS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical fatigue</td>
<td>7.0 (2.2)</td>
<td>8.1 (2.2)</td>
<td>9.3 (2.7)</td>
<td>8.9 (2.3)</td>
</tr>
<tr>
<td>Mental fatigue</td>
<td>4.0 (1.6)</td>
<td>4.0 (1.4)</td>
<td>8.1 (2.8)</td>
<td>7.6 (2.9)</td>
</tr>
</tbody>
</table>

Control (n=7), fish oil (n=7). Values are mean (SEM). Abbreviations: IPAQ, International Physical Activity Questionnaire; MET, metabolic equivalents of task; PFS, Pittsburgh Fatigability Scale.
Figure 5.4. Walking speed, heart rate, and tissue saturation index during the 400m walk test.

Control (n=8), fish oil (n=9). Abbreviations: bpm, beats per minute; TSI, tissue saturation index.
Height was then added as a covariable for heart rate analysis during the 400m walk test as there was an association between height and walking speed in this study and other studies (Bohannon 1997, Mikos, Yen et al. 2018). In addition, due to the probability of walking velocity change as participants get going in the first lap and approach the end and have energy left to expend in the final lap, these laps were discarded from estimates of steady state velocity and heart rate during the 400m walk test. There was a decrease in mean heart rate within the fish oil group pre to post during the 400m walk test (P<0.01), in addition to a difference between the control and fish oil groups post supplementation (P<0.01) (figure 5.5).

![Figure 5.5](image-url)

**Figure 5.5.** Heart rate collected before and after supplementation during the 400m walk test in the control (n=8) and fish oil (n=9) group.

*Mean heart rate during laps 2-9 (first and last lap excluded). Covariable; height. *P<0.01.*
Heart rate recovery following the 400m walk test was measured as the heart beat drop from peak within the first and second minute of seated rest. There were no significant differences in heart rate recovery between groups (P>0.05) (table 5.18).

**Table 5.18.** Heart rate recovery (heart beat drop) at 1 minute and 2 minutes following the 400m walk test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Fish Oil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>1 min</td>
<td>31 (2)</td>
<td>31 (2)</td>
<td>34 (3)</td>
<td>35 (3)</td>
</tr>
<tr>
<td>2 min</td>
<td>41 (3)</td>
<td>40 (3)</td>
<td>46 (3)</td>
<td>47 (2)</td>
</tr>
</tbody>
</table>

Control (n=8), fish oil (n=9). Values are mean (SEM).

5.9 Submaximal treadmill protocol

The submaximal treadmill protocol consisted of a 5 minute seated rest period prior to treadmill walking, followed by 2 walking stages. Treadmill speed was increased during stage 1. Treadmill incline was increased during stage 2. There were no significant differences in heart rate, net oxygen consumption, oxygen cost of walking, tissue saturation index or rating of perceived exertion at rest or during treadmill walking (P>0.05) (table 5.19).

Carbohydrate and fat oxidation rates, as well as the respiratory exchange ratio were not significantly different at rest or during treadmill walking (P>0.05) (table 5.20).
Table 5.19. Heart rate, oxygen consumption, oxygen cost of walking, tissue saturation index and rating of perceived exertion data collected during the submaximal treadmill protocol.

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (bpm)</th>
<th>Net oxygen consumption (mL/kg/min)</th>
<th>Oxygen cost of walking (mL/m)</th>
<th>Relative change in tissue saturation index (%)</th>
<th>Rating of perceived exertion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Fish Oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Resting</td>
<td>74 (2)</td>
<td>74 (3)</td>
<td>69 (3)</td>
<td>65 (2)</td>
<td>7 (0.6)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>113 (5)</td>
<td>111 (6)</td>
<td>106 (4)</td>
<td>102 (4)</td>
<td>10 (0.8)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>126 (7)</td>
<td>121 (8)</td>
<td>111 (5)</td>
<td>110 (5)</td>
<td>10 (0.8)</td>
</tr>
<tr>
<td>Resting</td>
<td>2.97 (0.28)</td>
<td>3.33 (0.43)</td>
<td>2.82 (0.27)</td>
<td>2.99 (0.26)</td>
<td>9 (0.6)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>13.64 (1.85)</td>
<td>11.46 (0.97)</td>
<td>10.11 (1.10)</td>
<td>12.01 (1.08)</td>
<td>10 (0.8)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>17.19 (1.58)</td>
<td>14.73 (1.82)</td>
<td>13.06 (0.58)</td>
<td>14.71 (0.95)</td>
<td>11 (0.9)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.32 (0.04)</td>
<td>0.27 (0.03)</td>
<td>0.26 (0.04)</td>
<td>0.31 (0.03)</td>
<td>12 (0.9)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.08 (0.01)</td>
<td>0.07 (0.01)</td>
<td>0.06 (0.01)</td>
<td>0.07 (0.01)</td>
<td>11 (0.9)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>-44.97 (6.16)</td>
<td>-37.46 (4.94)</td>
<td>-40.97 (3.27)</td>
<td>-38.00 (3.93)</td>
<td>11 (1.0)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>-51.96 (4.92)</td>
<td>-41.60 (5.10)</td>
<td>-49.10 (4.25)</td>
<td>-44.03 (5.03)</td>
<td>11 (1.6)</td>
</tr>
</tbody>
</table>

Resting and stage one heart rate and stage one tissue saturation index; control (n=8), fish oil (n=9). Resting oxygen consumption and stage one net oxygen consumption and oxygen cost of walking; control (n=7), fish oil (n=9). Resting and stage two heart rate and stage one tissue saturation index; control (n=7), fish oil (n=7). Stage two net oxygen consumption and oxygen cost of walking; control (n=6), fish oil (n=7). Abbreviations: bpm, beats per minute. Resting oxygen consumption expressed at gross oxygen consumption.
Table 5.20. Carbohydrate oxidation rate, fat oxidation rate and respiratory exchange ratio at rest and during stage one and stage two of treadmill walking.

<table>
<thead>
<tr>
<th></th>
<th>Carbohydrate use (g/min)</th>
<th>Fat use (g/min)</th>
<th>Respiratory exchange ratio (V\textsubscript{CO}_2/V\textsubscript{O}_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Fish Oil</td>
<td>Pre</td>
</tr>
<tr>
<td>Resting</td>
<td>0.15 (0.04)</td>
<td>0.31 (0.13)</td>
<td>0.21 (0.08)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.95 (0.23)</td>
<td>0.80 (0.15)</td>
<td>0.82 (0.12)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>1.42 (0.32)</td>
<td>1.17 (0.28)</td>
<td>1.07 (0.15)</td>
</tr>
</tbody>
</table>

Resting and stage one; control (n=7), fish oil (n=9). Stage two; control (n=6), fish oil (n=7). Abbreviations: V\textsubscript{CO}_2/V\textsubscript{O}_2, ratio between volume of carbon dioxide released and volume of oxygen consumed.
Heart rate recovery was then assessed for 10 minutes following exercise cessation with the participant laying supine in a dark room using a heart rate decay formula ($t_{1/2}$) which examined the half time for heart rate to drop back to resting. There was no difference detected between groups for half time recovery (table 5.21). Total heart beat drop at the end of the 10-minute supine period was not significantly different between groups post-supplementation (table 5.21).

**Table 5.21.** Heart rate recovery following the treadmill walking protocol.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fish Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Minimum HR (bpm)</td>
<td>58  (2)</td>
<td>58  (2)</td>
</tr>
<tr>
<td>Peak HR (bpm)</td>
<td>127 (6)</td>
<td>121 (6)</td>
</tr>
<tr>
<td>$t_{1/2}$ (s)</td>
<td>131 (16)</td>
<td>127 (16)</td>
</tr>
<tr>
<td>Total beat drop</td>
<td>68  (6)</td>
<td>63  (6)</td>
</tr>
</tbody>
</table>

_Control (n=8), fish oil (n=9). Abbreviations: HR, heart rate; bpm, beats per minute; $t_{1/2}$, time to half._
5.10 Ambulatory 24-hour heart rate monitoring

All participants completed the 24-hour heart rate monitoring. The mean (SEM) sample duration for the control group was 403 (35) minutes pre supplement and 494 (30) minutes post supplement. The mean (SEM) sample duration for the fish oil group was 507 (23) minutes pre supplement and 509 (19) minutes post supplement. There were no significant differences in sample duration between groups (P>0.05). There were no significant differences (P>0.05) for mean overnight heart rate or time, non-linear or frequency heart rate variability domains at baseline and following the supplementation period (table 5.22). There was a small, non-significant reduction (P=0.28) in heart rate change for the fish oil group (-2.2 beats) compared to control (-0.8 beats) (figure 5.6).

Figure 5.6. Heart rate change collected via a cardiac monitor during overnight sleep in control (n=8) and fish oil (n=9) groups. P=0.28.
Table 5.22. Overnight heart rate and heart rate variability parameters.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Fish Oil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Mean heart rate (bpm)</td>
<td>63 (2)</td>
<td>62 (2)</td>
<td>60 (2)</td>
<td>57 (2)</td>
</tr>
<tr>
<td>Night-day heart rate ratio</td>
<td>0.90 (0.04)</td>
<td>0.86 (0.04)</td>
<td>0.91 (0.04)</td>
<td>0.87 (0.05)</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>63.17 (5.07)</td>
<td>74.12 (9.73)</td>
<td>89.34 (8.25)</td>
<td>78.76 (5.26)</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>20.36 (2.82)</td>
<td>20.35 (3.30)</td>
<td>27.66 (5.94)</td>
<td>31.17 (6.53)</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>14.40 (2.00)</td>
<td>14.39 (2.33)</td>
<td>19.56 (4.20)</td>
<td>22.04 (4.62)</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>88.03 (7.34)</td>
<td>103.71 (13.69)</td>
<td>124.47 (11.39)</td>
<td>108.64 (6.99)</td>
</tr>
<tr>
<td>SD2/SD1</td>
<td>6.75 (0.87)</td>
<td>7.88 (1.03)</td>
<td>7.97 (1.04)</td>
<td>6.48 (1.01)</td>
</tr>
<tr>
<td>Power HF-band</td>
<td>162.42 (51.43)</td>
<td>165.51 (56.07)</td>
<td>247.50 (87.08)</td>
<td>322.04 (114.68)</td>
</tr>
<tr>
<td>Power LF-band</td>
<td>320.40 (60.68)</td>
<td>293.73 (81.42)</td>
<td>529.67 (136.96)</td>
<td>612.41 (151.69)</td>
</tr>
<tr>
<td>Power VLF-band</td>
<td>2065.30 (837.49)</td>
<td>1454.60 (235.17)</td>
<td>2077.10 (336.39)</td>
<td>1854.60 (289.13)</td>
</tr>
<tr>
<td>Power total</td>
<td>2548.10 (867.08)</td>
<td>1913.80 (337.35)</td>
<td>2854.20 (518.51)</td>
<td>2789.10 (500.60)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>2.83 (0.58)</td>
<td>2.92 (0.60)</td>
<td>3.34 (0.63)</td>
<td>3.16 (0.72)</td>
</tr>
</tbody>
</table>

Control (n=8), fish oil (n=9). Values are mean (SEM). Abbreviations: bpm, beats per minute; SDNN, standard deviation of normal to normal intervals; RMSSD, root mean square of the successive differences; SD1, standard deviation of points perpendicular to the axis of line-of-identity; SD2, standard deviation of points along the axis of line-of-identity; SD1/SD2, ratio of SD1 to SD2; HF-band, high frequency band; LF-band, low frequency band; VLF-band, very low frequency band; LF/HF, ratio of LF to HF bands.
During the 10 minute period of overnight sleep where heart rate was the lowest recorded, the heart rate difference pre to post in the fish oil group was significantly different (P<0.05) to the control group (figure 5.7). No significant differences (P>0.05) were seen for time, non-linear or frequency heart rate variability domains during the lowest 10 minute period (table 5.23).

**Figure 5.7.** Heart rate change during the lowest ten minute heart rate period overnight.  

*Control (n=8), fish oil (n=9). *P<0.05*
Table 5.23. Heart rate and heart rate variability parameters collected during the lowest ten minute heart rate period overnight.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Fish Oil</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Mean heart rate (bpm)</td>
<td>58 (2)</td>
<td>58 (2)</td>
<td>55 (2)</td>
<td>52 (2)</td>
</tr>
<tr>
<td>Night-day heart rate ratio</td>
<td>0.83 (0.03)</td>
<td>0.81 (0.03)</td>
<td>0.85 (0.03)</td>
<td>0.79 (0.04)</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>36.58 (8.40)</td>
<td>48.89 (6.28)</td>
<td>37.79 (4.73)</td>
<td>45.92 (6.97)</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>20.31 (3.83)</td>
<td>22.11 (4.68)</td>
<td>26.70 (4.58)</td>
<td>29.12 (5.70)</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>14.36 (2.71)</td>
<td>15.63 (3.31)</td>
<td>18.88 (3.24)</td>
<td>20.59 (4.03)</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>49.36 (11.78)</td>
<td>66.94 (8.72)</td>
<td>49.09 (6.75)</td>
<td>60.48 (9.88)</td>
</tr>
<tr>
<td>SD2/SD1</td>
<td>3.53 (0.45)</td>
<td>5.01 (0.85)</td>
<td>3.17 (0.61)</td>
<td>3.57 (0.84)</td>
</tr>
<tr>
<td>Power HF-band</td>
<td>187.68 (72.57)</td>
<td>203.54 (94.29)</td>
<td>262.84 (89.25)</td>
<td>260.92 (96.20)</td>
</tr>
<tr>
<td>Power LF-band</td>
<td>317.36 (118.25)</td>
<td>397.79 (129.29)</td>
<td>368.79 (74.38)</td>
<td>380.73 (108.08)</td>
</tr>
<tr>
<td>Power VLF-band</td>
<td>1052.20 (656.36)</td>
<td>1247.50 (393.83)</td>
<td>748.82 (227.02)</td>
<td>962.82 (262.66)</td>
</tr>
<tr>
<td>Power total</td>
<td>1557.30 (781.18)</td>
<td>1848.80 (533.94)</td>
<td>1380.50 (268.46)</td>
<td>1604.50 (388.38)</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.97 (0.36)</td>
<td>3.33 (0.89)</td>
<td>3.02 (0.65)</td>
<td>2.69 (1.01)</td>
</tr>
</tbody>
</table>

Control (n=8), fish oil (n=9). Values are mean (SEM). Abbreviations: bpm, beats per minute; SDNN, standard deviation of normal to normal intervals; RMSSD, root mean square of the successive differences; SD1, standard deviation of points perpendicular to the axis of line-of-identity; SD2, standard deviation of points along the axis of line-of-identity; SD1/SD2, ratio of SD1 to SD2; HF-band, high frequency band; LF-band, low frequency band; VLF-band, very low frequency band; LF/HF, ratio of LF to HF bands.
6. Discussion

In healthy older adults, a low dose of DHA-rich fish oil increased the omega-3 index, indicative of lower cardiovascular disease risk, and delivering physiological change, in particular to heart rate, that may be of benefit in an aging population.

6.1 Aged physiology

Participants in the current study would be classified as highly fit and high functioning older adults. This is based on their physical function measures compared to normative data based on age in a group of healthy older adults (Rikli and Jones 1999). During the sit to stand assessment participants achieved a total of 7 and 5 repetitions more than normative values for males and females, respectively (Rikli and Jones 1999). As it relates to the 30s arm curl assessment, participants achieved an additional 5 (male) and 2 (female) compared to normative values (Rikli and Jones 1999). Participants were also 22% (male) and 26% (female) faster during the timed up and go assessment compared with normative values (Rikli and Jones 1999). Rikli et al., (2013) determined the functional fitness values required to maintain physical independence during older life (Rikli and Jones 2013). Even with these higher reference values, participants in the current study exceeded the sit to stand values by 7 repetitions (male) and 4 repetitions (female), arm curl values by 5 repetitions (male) and 1 repetition (female), and were faster during the timed up and go by 25% (male) and 20% female). Taken together, this can be interpreted as participants in the current study being at the top end of physical function based on age and have not experienced a large decline in physical function with age, even when compared with healthy older adults.
In the current study, participants had a preferred walking speed at baseline of 1.26 m/s, surpassing speeds associated with higher mortality risk (<0.8 m/s) (Stanaway, Gnjidic et al. 2011, Studenski, Perera et al. 2011), lower extremity limitation and death (<1.0 m/s) (Cesari, Kritchevsky et al. 2005), in addition to exceeding the normative criteria for age of 1.19 m/s in a group of healthy adults (Bohannon and Williams Andrews 2011). Walking speeds over 1.2 m/s suggest life expectancy well above the average and it is here where the participants in the current study fit (Studenski, Perera et al. 2011).

Older adults who have a slower walking speed display higher fatigue over 24 hours and overall perform less physical activity as the day progresses (Schrack, Simonsick et al. 2010, Schrack, Zipunnikov et al. 2014). Even in a high functioning group of older adults, this physiological process was evident in the current study. Walking speed collected during the 20m and 400m walk tests was inversely associated with the oxygen cost of walking on the treadmill, that is, the slower walkers also had the highest oxygen cost of walking on the treadmill. This supports Wert et al., (2013) that, when measured separately, found an inverse association between walking speed and energy cost of walking, as in the current study (Wert, Brach et al. 2013).

As it relates to oxygen cost of walking at varying treadmill speeds and gradients, the current study found that participants had a higher relative oxygen cost of walking at the slower treadmill speeds, however as the treadmill speed increased and they approached their usual walking speed there was a decrease in the relative oxygen cost of walking. This is in line with the literature, in that there is a U-shaped relationship between the oxygen cost of walking and walking speed, whereby oxygen cost of walking is higher at very low and very high walking speeds. The optimal speed or
economical speed is the walking speed which minimises the energy cost of walking (Abe, Fukuoka et al. 2015). As in the current study, Schrack et al., (2016) also found an inverse association between walking speeds and energy cost of walking in older adults (Schrack, Zipunnikov et al. 2016).

6.2 Long-chain omega-3 polyunsaturated fatty acids: intake and supplementation

6.2.1 Determination of omega-3 status

The current study used the omega-3 index as the primary outcome describing the omega-3 status of participants. In the current study, neither the whole blood LC n-3 PUFA nor the omega-3 index aligned well with either of the dietary assessments of omega-3 intake. This questions the validity of these short term measures for establishing habitual intake for individuals and demonstrates the importance of using direct measures of incorporation, such as the omega-3 index, to link to physiological or clinical effect. This was further highlighted by the large variations in fat intake reported in the 3-day food record.

The original development and validation of the PUFA FFQ in estimating blood levels of LC n-3 PUFA was performed in a relatively young group of adults with a maximum age of 58 years old (Sullivan, Williams et al. 2006, Sullivan, Brown et al. 2008). The PUFA FFQ used in the current study was the electronically administered version which has been validated in a relatively small sample of adults, including older adults (Swierk, Williams et al. 2011). Although older adults aged 59-79 years (n=12) were included during the development and validation of the electronically administered PUFA FFQ, a separate analysis was not performed on that age group and therefore the inclusion of all ages may limit the applicability to older adults alone. The PUFA FFQ is reported to capture dietary intake of previous 3 months which is an important consideration when
investigating foods which are consumed infrequently, such as fish. Despite Swierk et al., (2011) reporting that the PUFA FFQ adequately estimates blood levels of LC n-3 PUFA across a wide age range, the current study did not reflect these findings.

The lack of agreement between the 3-day food record and blood levels of LC n-3 PUFA is not surprising. Fish consumption does not often occur on a daily basis and the possibility for underreporting fish intake is higher. Nonetheless, dietary data from the 3-day food record was not collected for the purpose of estimating blood levels of LC n-3 PUFA, but rather to establish no change in nutrient or energy intake throughout the study or between groups. Due to the lack of agreement between self-reported dietary measures and objective blood markers, it highlights the importance of measuring circulating blood biomarkers such as the omega-3 index before and after supplementation to objectively evaluate the effect of LC n-3 PUFA on outcome measures. In addition, the use of the omega-3 index, which is the content of EPA + DHA in RBC membranes, expressed as a percent of total fatty acids (Harris and Von Schacky 2004), is more representative of chronic fish intake compared with plasma (Harris, Varvel et al. 2013). Considerations when designing omega-3 clinical trials have been addressed (Rice, Bernasconi et al. 2016). These considerations include an adequate dose of long-chain omega-3 fatty acids to see an effect in primary outcome measures, an appropriate study population with low baseline omega-3 status, and measurement of omega-3 status at baseline and during treatment to ensure low omega-3 levels at baseline and to assess compliance. In the present study, the omega-3 index (Harris and Von Schacky 2004) was used to screen out participants with a high omega-3 index at baseline and appropriately match participants into the control and fish oil groups to prevent one group from having a significantly different omega-3 status at baseline.
6.2.2  *Dose and composition of supplemental omega-3 fatty acids*

The current study showed that low dose DHA-rich fish oil supplemented for 16 weeks significantly increased the omega-3 status from baseline, elevating the omega-3 index over the desired 8% which has been suggested for optimal cardio protection (Harris and Von Schacky 2004). The dose used in the current study is in line with the current Australian Dietary Guidelines of approximately 2 fish meals per week (National Health and Medical Research Council 2013). Dietary fish oil incorporation of LC n-3 PUFA into skeletal and cardiac muscle has been examined in animal models (Ayre and Hulbert 1996, Pepe and McLennan 1996, Peoples and McLennan 2010, Peoples and McLennan 2014). There is preferential incorporation of DHA into skeletal muscle when supplemented with DHA-rich fish oil (Peoples and McLennan 2010) and this occurs even when an EPA-rich fish oil source is used (Pepe and McLennan 1996). In rats, DHA is preferentially incorporated into heart and skeletal muscle with little change in EPA levels (Owen, Peter-Przyborowska et al. 2004). Incorporation of LC n-3 PUFA into skeletal and cardiac muscle is also evident at lower doses, whereby DHA in rats is preferentially incorporated into type 2 muscle fibers (Henry, Peoples et al. 2015), and myocardial membranes (Slee, McLennan et al. 2010) following low dietary doses of DHA-rich fish oil. Changes in erythrocytes show a similar relationship with higher DHA incorporation in dogs (Stoeckel, Nielsen et al. 2011). The findings from animal models have been further evaluated in humans. As in the animal models, LC n-3 PUFA successfully incorporate into human cardiac (Metcalf, James et al. 2007) and human skeletal muscle membranes (Andersson, Nalsen et al. 2002, Haugaard, Madsbad et al. 2006, McGlory, Galloway et al. 2014).
The finding of an elevated omega-3 index in the current study was achieved through low dose DHA-rich fish oil supplementation. The measurement of erythrocyte fatty acids are reflective of EPA+DHA content in human cardiac tissue (Harris, Sands et al. 2004, Metcalf, Cleland et al. 2010) and human skeletal muscle tissue (Andersson, Nalsen et al. 2002). In the current study, the increase in the omega-3 index was primarily driven by DHA, rather than EPA. This supports work done in rodents, showing that DHA incorporates to a greater extent than EPA within muscle tissues (Slee, McLennan et al. 2010, Henry, Peoples et al. 2015). Milte et al., (2008) supplemented a DHA-rich fish oil in middle aged adults at DHA doses ranging from low (520mg/d) to high (1560mg/d). In agreement with the current study, it was found that all doses caused a significant increase in erythrocyte EPA+DHA content, however it was DHA that drove the increase in erythrocyte EPA+DHA at all doses, rather than EPA (Milte, Coates et al. 2008). This is supported further by the findings of Theobald et al., (2004) who supplemented middle aged adults with low dose DHA which caused a significant increase in erythrocyte DHA, whereas EPA remained unchanged (Theobald, Chowienczyk et al. 2004). In addition to Allaire et al., (2017) which established that when EPA and DHA are supplemented individually, it is DHA which caused the greater increase in the omega-3 index compared with EPA in middle aged adults (Allaire, Harris et al. 2017). Flock et al., (2013) found a similar result whereby DHA increases more than EPA within erythrocytes, however in this study an EPA-rich fish oil capsule was used, highlighting the preferential incorporation of DHA over EPA irrespective of a higher supplemental dose of EPA (Flock, Skulas-Ray et al. 2013).

Other studies in older adults have predominantly used high doses of fish oil, with EPA-rich fish oil capsules (Smith, Atherton et al. 2011, Rodacki, Rodacki et al. 2012, Hutchins-Wiese,
Kleppinger et al. 2013, Krzymińska-Siemaszko, Czepulis et al. 2015, Monahan, Feehan et al. 2015, Smith, Julliand et al. 2015, Clark, Monahan et al. 2016, Da Boit, Sibson et al. 2017, Alfaddagh, Elajami et al. 2018, Clark, Monahan et al. 2018). Not only does the high dose make it difficult to reproduce through dietary sources, but the higher proportion of EPA to DHA in most studies are also not representative of fatty acid profiles of fresh fish, which are often higher in DHA rather than EPA (Soltan and Gibson 2008, Mozaffarian and Wu 2011). The current study differed from other studies in older adults in that participants were supplemented with a low dose of DHA-rich fish oil, which could be reproduced by dietary intake of approximately 2 fatty fish meals per week, yet it still established a significant increase in the omega-3 index, driven by DHA. To date, increases in erythrocyte EPA+DHA following low dose DHA-rich fish oil supplementation, reflective of fresh fish (Soltan and Gibson 2008, Mozaffarian and Wu 2011), have been established in healthy young (Macartney, Hingley et al. 2014, Hingley, Macartney et al. 2017) and middle-aged adults (Theobald, Chowienczyk et al. 2004, Milte, Coates et al. 2008). The current study adds to these findings by establishing the effect of a low dose of DHA-rich fish oil in elevating the omega-3 index in older adults.

6.3 Effect of fish oil on cardiovascular outcomes at rest and exercise

Fish oil consumption successfully reduced heart rate during fast paced walking over 400m, and this occurred without a change in physical work. This indicates that submaximal heart rate can be affected in older adults, as in healthy, well trained young adults and with the use of much lower fish oil intakes than were previously used (Peoples, McLennan et al. 2008, Buckley, Burgess et al. 2009).
As it relates to older adults, Logan et al., (2015) found that high dose EPA-rich fish oil supplementation for 12 weeks reduced submaximal heart rate by 2 beats during low intensity cycling in older females. In contrast, the dose of fish oil used by Logan et al., (2015) was higher in EPA compared to DHA and approximately 4 times greater than what was used in the current study. The heart rate reduction in the current study of 7 beats is higher to what was reported by Logan et al., (2015), however the exercise intensity used by Logan et al., (2015) was very low (40% of heart rate reserve) and may not have been sufficient to see a greater effect of the heart rate lowering caused by fish oil supplementation.

Macartney et al., (2014) supplemented healthy males with the same low dose of DHA-rich fish oil used in the current study. Following 8 weeks of supplementation, participants achieved a submaximal heart rate reduction of 4.5 beats which is in line with what was found in the current study. The current study is the first study to establish that a low dose of DHA-rich fish oil successfully reduces submaximal exercise heart rate in a group of healthy, active older adults.

In contrast to the heart rate lowering effects found in the 400m walk test, a lowered heart rate was not observed during the treadmill walking assessment, where heart rate reached approximately 75% of age-predicted maximum, compared to the 85% of age-predicted maximum in the 400m walk. There are several possibilities that may have contributed to this outcome. It was noted that the influence of walking instability on the treadmill and anxiety during treadmill speed changes caused heart rate to fluctuate to a greater extent than during over ground walking, often causing elevations in heart rate not explainable by work load, despite participants being familiarized with the treadmill protocol. This was confirmed by the participants perceived rating of exertion.
corresponding to ‘light’ activity during the most difficult stage of the treadmill walk. This finding of an elevated heart rate, not explained by work rate, in older adults during treadmill walking has been documented in other studies (Swerts, Mostert et al. 1990, Greig, Butler et al. 1993). When comparing to corridor walking, Greig et al., (1993) found that treadmill walking, at equal speed, resulted in an elevated heart rate in the elderly. The use of a mouthpiece, which was used on treadmill but not over ground walking also affected heart rate. The current study did not use equivalent walking speeds between treadmill walking and over ground walking assessments, however a mouth piece was used to collect oxygen consumption on the treadmill. There are a number of potential reasons which may explain the unexpected heart rate response to treadmill walking in the elderly, including fear of falling, altered stride length and altered cadence (Peeters and Mets 1996, Wass, Taylor et al. 2005, Marsh, Katula et al. 2006). Taken together, the adjustments which occur during treadmill walking seem to reflect instability and lack of balance which influence heart rate responses to treadmill walking. Therefore, the 400m walking test appears to more accurately reflect free living conditions in older adults and it is here where fish oil was found to lower heart rate.

The current study showed that in the context of highly active, healthy older adults, the low dose of DHA-rich fish oil also reduced minimum overnight heart rate. The effects of fish oil feeding in reducing intrinsic heart rate is evident in human cardiac transplant patients (Harris, Gonzales et al. 2006), and in animal models (Pepe and McLennan 2002, Dhein, Michaelis et al. 2005, Abdukeyum, Owen et al. 2008, Verkerk, den Ruijter et al. 2009) where vagal tone is not present. Verkerk et al., (2009) established that the prolonged cardiac cycle length found in rabbits fed a diet enriched with fish oil was a result of a reduction in pacemaker current density in isolated sino-
atrial node cells. These intrinsic effects of fish oil on heart rate are at least in part due to the ability of polyunsaturated fatty acids in modulating myocardial calcium handling and preventing spontaneous calcium release (Negretti, Perez et al. 2000, Leifert, Dorian et al. 2001, Sankaranarayanan and Venetucci 2012). The current study supports the hypothesis of a direct effect of fish oil in lowering intrinsic heart rate because overnight heart rate variability, a measure of autonomic function, was unchanged as a result of fish oil supplementation, suggesting that the reduction in minimum overnight heart rate occurred in the absence of either increased parasympathetic nervous system activity, or decreased sympathetic nervous system activity. Additionally, the nocturnal dip that occurs during overnight sleep is also an indicator of autonomic function and is related to sympathetic over activity. DHA-rich fish oil did not have an effect on the ratio between night-day heart rate in the current study. Taken together, this implies that low dose DHA-rich fish oil had a direct effect on intrinsic heart rate, as all measures relating to autonomic function were not influenced by fish oil supplementation.

Despite the importance and clinical usefulness of overnight heart rate, little work has been done surrounding fish oil’s effects on overnight heart rate lowering. There is evidence in children that fish oil lowers overnight heart rate (Buchhorn, Willaschek et al. 2017), however the current study is the first to provide evidence that a low dose of fish oil lowers intrinsic heart rate during overnight sleep in healthy, active older adults.

Up to this point in time the primary method for selective heart rate lowering has been through pharmacological intervention (Bruguera Cortada and Varela 2009, Tardif 2009, Rosano, Vitale et al. 2010), however the reduction in heart rate, both at rest and exercise, often decreases heart rate reserve and exercise capacity during exercise (Van Baak 1988, Van Bortel and Van Baak 1992, Vanhees, Defoor et al. 2000). There is strong evidence to support the heart rate lowering effects of fish oil (Mozaffarian, Geelen et al. 2005), however in contrast to the heart rate lowering achieved with pharmacological intervention, the lowering of heart rate achieved with fish oil does not reduce cardiovascular function or exercise capacity and under some conditions can even serve to improve it (Peoples, McLennan et al. 2008, Macartney, Hingley et al. 2014, Hingley, Macartney et al. 2017).

The association of reduced heart rate with an elevated omega-3 index is novel. The omega-3 index has predominantly been investigated for its role in overall cardiovascular disease risk (Harris 2008), whereas in the current study it was found that elevating the omega-3 index above 8% resulted in a reduction in heart rate at rest and during exercise. Notably, elevated heart rate is a significant contributor to risk of mortality (Palatini, Casiglia et al. 1999, Seccareccia, Pannozzo et al. 2001, Jouven, Empana et al. 2005, Zhang, Shen et al. 2016). A recent meta-analysis found a
reduction in resting heart rate of approximately 2.5 beats with fish oil supplementation (Hidayat, Yang et al. 2017), and the results from the current study are in line with these findings. The significance of a 3-4 beat per minute reduction in heart rate as seen in the current study on a lower sudden death risk appears to be approximately 7-10% on the basis of Jouven et al., (2005) and a 10% decrease in cardiovascular death and hospital admission for worsening heart failure (Bohm, Swedberg et al. 2010). Not only is a lower resting heart rate advantageous for reducing risk of all-cause (Seccareccia, Pannozzo et al. 2001, Jouven, Empana et al. 2005, Zhang, Shen et al. 2016) and cause-specific mortality (Palatini, Casiglia et al. 1999, Fox, Borer et al. 2007), but more recently the significance of a consistently low resting heart rate over time has been highlighted as an independent risk factor for all-cause mortality (Wang, Li et al. 2017). As the consumption of fish and fish oil supplementation incorporates LC n-3 PUFA, in particular DHA, into myocardial membranes there is opportunity for long term physiological remodeling and thus a consistent heart rate lowering effect. Future research should aim to determine if this is also part of the cardiac protective effects when fish is consumed in the diet.

6.4 Effect of fish oil on physical function and strength measures

In the current study, fish oil supplementation did not change physical function or strength measures, however a small positive correlation was found between pinch grip strength and the omega-3 index at baseline within the entire cohort. There is a lack of consistency between findings of increased strength measures from fish oil supplementation. It seems that EPA-rich fish oil primarily serves to augment muscle strength increases, only when combined with a resistance training program (Rodacki, Rodacki et al. 2012, Da Boit, Sibson et al. 2017), whereas the current study did not combine DHA-rich fish oil supplementation with an exercise intervention, but rather recruited physically active older adults who did not change their exercise regimes. Smith et al.,
(2015) found a significant increase in muscle strength after high dose EPA-rich fish oil supplementation without a combined resistance training program, however the participants in the control group decreased their muscle strength to a similar extent as the fish oil group increased muscle strength. Smith et al., placed no control over physical activity or dietary behaviors throughout the intervention and this may explain their unexpected finding. Other studies have found no effect of fish oil on muscle strength in older adults with decreased muscle mass (Krzymińska-Siemaszko, Czepulis et al. 2015) or who met pre-frail or frail criteria (Hutchins-Wiese, Kleppinger et al. 2013).

When assessed for walking speed, participants in the current study far exceeded values associated with mortality risk (Abellan van Kan, Rolland et al. 2009, Stanaway, Gnjidic et al. 2011), lower extremity limitation, hospitalisation and death (Cesari, Kritchevsky et al. 2005), as well as the normative values for age (Bohannon and Williams Andrews 2011). Fish oil supplementation did not alter walking speed in the current study. Older adults who have a slower walking speed display higher fatigue over 24 hours and overall perform less physical activity as the day progresses (Vestergaard, Nayfield et al. 2009, Schrack, Zipunnikov et al. 2014). Large variability between individuals exists as to the extent of muscle mass and physical function loss with age. Modifiable physical activity and nutrition behaviors largely explain the variability in the rate of muscle mass and physical function loss (Witard, McGlory et al. 2016). The participants in the current study were highly active, healthy older adults. Participants reported on average a total of 79 minutes per day of moderate intensity physical activity in the International Physical Activity Questionnaire which is more than double the Australian Government recommendation of 30 minutes on most, preferably all, days. In addition, participants reported very low mental and physical fatigue in the
Pittsburgh Fatigability Scale. The Pittsburgh Fatigability Scale is designed to measure perceived fatiguability in older adults across a range of day to day tasks and ranges from 0-50, whereby a higher score represents high fatiguability. Participants reported mean scores under 10 in both physical and mental fatigue, indicating a very low fatiguing cohort. Taken together, the high reported physical activity, and low reported fatigability, in addition to the high walking speed of the participants, it is not surprising that further improvements were not seen in walking speed or physical function measures. The effects fish oil on walking speed appear to only be evident in slow walkers with walking speeds under approximately 1.0 m/s. Data from the Three-City-Bordeaux study did show an association between high plasma concentrations of LC n-3 PUFA and higher walking speed in 982 adults aged 65 years and over (Frison, Boirie et al. 2015), however walking speed in this group was low, at below 1.0 m/s. Conversely, in participants with a walking speed over 1.0 m/s, Fougere et al., (2018) found no difference in walking speed of older adults at baseline between participants in the lowest quartile of omega-3 status compared to those in the upper three quartiles. Interventions involving omega-3 fatty acids have found similar results. In a group of older aged female participants who met pre-frail or frail criteria and displayed walking speed around 1.0m/s, fish oil was able to significantly increase walking speed, however the increase was small (0.03m/s) (Hutchins-Wiese, Kleppinger et al. 2013). There is a consistent lack of effect of high dose EPA-rich fish oil supplementation on walking speed in randomised control trials where older adults walk faster than 1.0m/s (Rodacki, Rodacki et al. 2012, Da Boit, Sibson et al. 2017).

6.5 Effect of fish oil on oxygen cost of exercise

During the treadmill walking protocol, the current study found no effect of fish oil supplementation on oxygen consumption or oxygen cost of walking. There is limited research around the role of omega-3 fatty acids in oxygen modulation and oxygen cost of walking in older adults. In contrast
to the finding of no change in the current study, Logan et al., (2015) reported an increase in oxygen consumption from high dose fish oil supplementation in older adults. This is surprising due to the role of omega-3 fatty acids in oxygen modulation within muscle, more specifically improving the efficiency of oxygen use, reflected in reduced whole body oxygen consumption in well trained cyclists (Peoples, McLennan et al. 2008). The ability of LC n-3 PUFA to incorporate into cardiac tissue is also shared with skeletal muscle tissue (Andersson, Nalsen et al. 2002, Owen, Peter-Przyborowska et al. 2004, McGlory, Galloway et al. 2014), and therefore the observed effects of a more efficient use of oxygen as a result of LC n-3 PUFA incorporation within the heart are also present within skeletal muscle as shown in animal models (Peoples and McLennan 2010, Peoples and McLennan 2014, Henry, Peoples et al. 2015). A lower skeletal muscle oxygen consumption was required for tension development in rats fed a fish oil diet (Peoples and McLennan 2014). The findings of a lower oxygen consumption from fish oil feeding in animal models has been investigated in humans, whereby a lower oxygen consumption is achieved with fish oil supplementation in aerobically trained (Peoples, McLennan et al. 2008, Hingley, Macartney et al. 2017) and untrained males (Kawabata, Neya et al. 2014). The increase in oxygen consumption reported by Logan et al., (2015) comes without an increase in workload, therefore suggesting a less efficient use of oxygen, which is not consistent with other animal or human evidence (Peoples, McLennan et al. 2008, Peoples and McLennan 2010, Kawabata, Neya et al. 2014, Peoples and McLennan 2014, Henry, Peoples et al. 2015, Hingley, Macartney et al. 2017). In fact, in other trials performed from the same lab as Logan et al., (2015) they have found either variable effects (Gerling, Whitfield et al. 2014) or no effect (Jannas-Vela, Roke et al. 2017) of fish oil supplementation on oxygen consumption in healthy recreationally active young (<25 years) males. Nonetheless, the finding of no change in oxygen consumption or oxygen cost of walking in the
The current study may be related to the aforementioned walking instability and anxiety during treadmill speed changes which made it difficult to represent an accurate heart rate response. Oxygen consumption is heavily influenced by heart rate during exercise (Bernard, Gavarry et al. 1997) and therefore due to heart rate being influenced by factors not relating only to work load during the treadmill protocol it may not provide an accurate representation of oxygen cost of walking or oxygen consumption for the given workloads. Perhaps a portable metabolic analyser which can be used during self-paced walking would provide a representation of oxygen consumption which is more reflective of free living conditions.

The effect of fish oil supplementation on lowering oxygen consumption during exercise is best evident under high external workloads causing a high physiological challenge (Peoples, McLennan et al. 2008, Kawabata, Neya et al. 2014, Hingley, Macartney et al. 2017). Participants in the current study were only exercising at approximately 75% of their age predicted maximum during the treadmill protocol and walking at a speed of approximately 65% of their over ground walking speed. Moreover, their responses across the range of physical assessments suggests they may have a higher than average maximum heart rate for age and therefore a greater physical activity reserve. The decline in physical function, muscle mass, and strength with age is highly variable. It is now known that habitual exercise training has the ability to strongly limit the age-associated decline in VO\textsubscript{2max}, peak strength and muscle mass (Wroblewski, Amati et al. 2011), as found in a recent meta-analysis comparing these parameters in master’s athletes (McKendry, Breen et al. 2018). It was found that master endurance athletes have comparable VO\textsubscript{2max} values to healthy young controls and above the values achieved in healthy, but less active older adults. It was also found that master strength/power athletes were stronger than the older age controls (McKendry, Breen et
al. 2018). Clearly, there is a notable gap in the rate of decline between individuals who are physically capable and those who are not.

6.6 Summary

Using a randomised placebo-controlled design, the aim of this study was to investigate the ability of low dose DHA-rich fish oil to modify cardiovascular function and physical performance, on the basis of an elevated omega-3 index. Careful screening included blood analysis of omega-3 status to ensure participants were not consuming fish or fish oil at a level that might obscure the effects of a low dose supplement, in addition to representing the median Australian LC n-3 PUFA intake or below. This resulted in a group of fit, healthy and active older adults, where the oxygen cost of exercise is still inherently linked to their walking speed. The strengths of this study included the randomised placebo-controlled design which had strict inclusion criteria to ensure participants were highly active to allow for repeatable physical performance at each visit and to minimize the chance of day to day performance variation as often seen in older adults.

6.7 Limitations

The participants in the current study were highly active older adults who displayed physical fitness, often in the highest functional categories for age. This allowed participants to successfully complete all the physical assessments required in the study, however it also meant that further improvement was less likely. Furthermore, due to the high physical performance of participants in the current study, the physical function assessments, such as timed up and go, sit to stand and the 400m walk test did not provide a strong physiological stress, whereas these tests are more demanding in older adults with lower physical function.
The current study included multiple visits with tightly controlled in-laboratory physiological tests, in addition to a range of functional and at-home assessments. This increased the participant burden which, in turn, made it difficult to achieve larger participant numbers. Potential participants were often limited by transport and having to travel to the University for several visits. The small sample size is a limitation of the current study. It is likely that measures such as heart rate recovery which approached significance, may have achieved significance if participant numbers were higher.

Despite fish oil supplementation successfully elevating the omega-3 status, participants still had a baseline omega-3 index of 6% which is not within the undesirable range for overall cardiovascular disease risk.

6.8 Recommendations for future studies
Future studies should investigate the role of low dose DHA-rich fish oil in modifying the oxygen cost of exercise and improving physical function, in particular walking speed, either (i) in a more fatigable cohort, where it is evident that participants have experienced notable age-associated declines in physical function, or (ii) within a highly active group of older adults as in the current study, however using more sensitive measures to capture muscle fatigue differences, such as using ultrasound images to detect muscle architecture (sonomyography) which has successfully been used to study muscle fatigue (Shi, Zheng et al. 2007). Using physical function measures which are more physiologically demanding, such as isokinetic and isometric maximum voluntary contraction (MVC) in a highly active and high functioning cohort may allow for further improvement in outcome measures as a result of supplementation. If a more fatigable cohort is used, future studies may benefit from placing a greater focus on functional assessments such as corridor walking, which can be completed at local recreation facility where older adults regularly visit, such as lawn
bowl clubs and tennis centres. In addition, recruiting participants with a lower omega-3 status at baseline may allow for further improvement in outcome measures.

6.9 Conclusion

There were three fundamental outcomes of the current study; First, confirming hypothesis one, a DHA-rich fish oil supplement (2g/day) did significantly modify the whole blood and specifically the erythrocyte membranes and reflected LC n-3 PUFA DHA in the diet. As a consequence, the omega-3 index was elevated into the low risk category for cardiovascular disease in this group of active older adults.

Second, confirming hypothesis two, an increased omega-3 index had demonstrable effects on cardiovascular function at rest and during exercise, namely a reduction in heart rate during overnight sleep and also during the 400m walking assessment. The extent of heart rate reduction is in line with what has been reported in population studies and meta-analysis. In fact, these reductions were observed independent of either increased parasympathetic nervous system activity or decreased sympathetic nervous system activity as demonstrated by unaltered heart rate variability and nocturnal dip. Therefore, this suggests a direct action of DHA-rich fish oil on the heart to lower intrinsic beat rate.

Third, DHA-rich fish oil supplementation did not modify the oxygen cost of exercise during treadmill walking, or walking speed during usual or rapid walking tests. Additionally, physical function measures, including grip strength, were not improved after supplementation. Therefore, in a group of healthy older adults, hypothesis three was rejected for this population of older adults.
7. References


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