

**DHA-RICH FISH OIL AND REGULAR MODERATE EXERCISE:
A COMBINED INTERVENTION TO IMPROVE CARDIOVASCULAR,
METABOLIC AND INFLAMMATORY BIOMARKERS IN OBESITY**

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ABSTRACT

The current obesity epidemic has intensified research on lifestyle interventions aimed at combating obesity and associated cardiovascular (CV) and metabolic risk. This clustering of risk factors with obesity is known as the “Metabolic Syndrome” (MS). There is now a large body of evidence detailing the ability of omega-3 fatty acids (n-3 FA) and regular moderate exercise to independently ameliorate several CV risk factors; however the combination of these interventions may be a more effective strategy in reducing CV risk than either treatment alone. This thesis describes the independent and combined effects of supplementation with docosahexaenoic acid (DHA) rich fish oil, and regular moderate exercise, on CV, metabolic and inflammatory biomarkers.

Sedentary, overweight volunteers (BMI > 25kg/m²) with mild hypertension (140/90 – 160/100mmHg), elevated plasma triglycerides (TAG) (>1.6mmol/L) or elevated total cholesterol (TC) (>5.5mmol/L) were recruited in three cohorts for a 12-week intervention trial. Subjects were randomised to one of the following interventions: fish oil, fish oil and exercise, sunflower oil (placebo), sunflower oil and exercise. Subjects consumed 6 g/day of DHA-rich fish oil (26% DHA, 6% EPA; ~1.9g n-3 FA) or sunflower oil. The exercise groups walked 3 days/wk for 45 min, at 75% age-predicted maximal heart rate (HR). Outcome measures were assessed and compared across each intervention group at Weeks 0, 6 and 12, with the exception of body composition, heart rate variability (HRV) and immune functions, which were assessed at Weeks 0 and 12 only. Apart from the consumption of allocated capsules, all subjects were instructed to maintain their normal diet during the study. If not asked to exercise as part of the intervention subjects were also instructed to maintain their normal level of physical activity.

Supplementation with DHA rich fish oil resulted in substantial increases in total long chain n-3 FA and DHA levels in erythrocyte membranes, accompanied by reduction of TAG, increase of high-density lipoprotein (HDL) cholesterol and reduction of superoxide production by stimulated neutrophils. Both the increase in HDL and the decrease in superoxide production were correlated with the change in erythrocyte DHA. Endothelium dependent arterial vasodilation (assessed by flow-mediated dilatation, FMD), HRV and HR response to exercise were also improved in subjects supplemented with the DHA-rich fish oil. Regular moderate intensity exercise, either alone or in addition to the DHA-rich fish oil supplementation, had no effect on these parameters, although it improved the compliance of small resistance arteries. Interestingly, however, both DHA-rich fish oil and regular exercise reduced body fat and these effects were additive when the interventions were combined. The change in fat mass was accompanied by an increase in fat oxidation during exercise, as measured by the respiratory exchange ratio. For the population as a whole, reductions in total and abdominal fat mass were associated with reductions in blood pressure.

In summary, this study is the first to evaluate the metabolic and CV benefits that can be achieved by combining n-3 FA supplementation from fish oil and regular aerobic exercise in overweight/obese adults. While this combination did not produce any synergistic effects, several independent benefits were attained. The high compliance rate (>85%) within this study indicates that this intervention is well tolerated and may therefore be sustainable in the longer term. Future research should evaluate the mechanisms underlying the n-3 FA - mediated improvements in body composition.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to a copy of my thesis being made available in the University Library.

Alison M Hill

31st March 2007

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GLOSSARY OF ABBREVIATIONS

AA	Arachidonic Acid
ACSM	American College of Sports Medicine
AHA	American Heart Association
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
Apo B	Apolipoprotein B
ATP III	Adult Treatment Panel III
β -receptor	Beta-Adrenoreceptor
BIA	Bioelectrical Impedance
BMI	Body Mass Index
BP	Blood Pressure
$^{\circ}$ C	Degrees Celsius
Ca ⁺⁺	Calcium
CDC	Centers for Disease Control and Prevention
CHD	Coronary Heart Disease
Con A	Concanavalin A
COX	Cyclooxygenase
CPT-1	Carnitine Palmitoyl Transferase-1
CRP	C-Reactive Protein
CV	Cardiovascular
CVD	Cardiovascular Disease
DAG	Diacylglycerol
DART	Diet and Reinfarction Trial
DBP	Diastolic Blood Pressure

DHA	Docosahexaenoic Acid
DPA	Docosapentaenoic Acid
DXA	Dual Energy X-ray Absorptiometry
ECG	Electrocardiogram
EDTA	Ethyldiaminetetraacetic Acid
ELISA	Enzyme Linked Immunoabsorbent Assay
eNOS	Endothelial NO Synthase
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FAT/CD 36	Fatty Acid Translocase
FMD	Flow-Mediated Dilatation
fMLP	f-met-leu-phe
FXR	Farnesol X Receptor
FO	Fish Oil
FOX	Fish Oil + Exercise
g	Gram
GTN	Glyceryl Trinitrate
GTN-D	Glyceryl - Trinitrate Mediated Dilatation
HbA _{1c}	Glycosylated Hemoglobin
HBSS	Hanks' Balanced Salt Solution
HDL	High Density Lipoprotein
HFP	High Frequency Power
HNF-4 α	Hepatic Nuclear Factor-4 α
HOMA	Homeostasis Model Assessment
HPEPE	Hydroperoxyeicosapentaenoic Acid
HPETE	Hydroperoxyeicosatetraenoic Acid

HR	Heart Rate
HRV	Heart Rate Variability
IAAT	Intra Abdominal Adipose Tissue
ICAM-1	Intracellular Adhesion Molecule-1
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IFN γ	Interferon Gamma
IGT	impaired glucose tolerance
IL-1 β	Interleukin 1 Beta
IL-1ra	Interleukin 1 receptor agonist
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
ISAK	International Society for the Advancement of Kinanthropometry
kg	Kilogram
kJ	Kilojoule
K ⁺	Potassium
LA	Linoleic acid
LNA	Alpha-linolenic acid
LDL	Low Density Lipoprotein
LAC	Large Artery Compliance
LFP	Low Frequency Power
LOX	Lipoxygenase
LPL	Lipoprotein Lipase
LPS	Lipopolysaccharide
LT	Leukotriene

LTA ₄	Leukotriene A ₄
LTA ₅	Leukotriene A ₅
LTB ₄	Leukotriene B ₄
LTB ₅	Leukotriene B ₅
LTC ₄	Leukotriene C ₄
LTC ₅	Leukotriene C ₅
LTD ₄	Leukotriene D ₄
LTD ₅	Leukotriene D ₅
LTn	Lymphotoxin
LXR	Liver X receptor
mg	Milligram
mL	Millilitre
mm	Millimetre
mRNA	Messenger Ribonucleic Acid
MAP	Mean Arterial Pressure
MI	Myocardial Infarction
MIP	Macrophage Inflammatory Protein
MNL	Mononuclear
MS	Metabolic Syndrome
n-3 FA	Omega-3 FA
n-6 FA	Omega-6-FA
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NATA	National Association of Testing Authorities of Australia
NBT	Nitroblue Tetrazolium
NCEP	National Cholesterol Education Program
NF-κB	Nuclear Factor-Kappa-Beta
NHMRC	National Health and Medical Research Council

NNS95	1995 Australian National Nutrition Survey
NO	Nitric Oxide
OD	Optical Density
OGTT	Oral Glucose Tolerance Test
PA	Physical Activity
PAI-1	Plasminogen Activator Inhibitor-1
PBMC	Peripheral Blood Mononuclear Cells
PG	Prostaglandin
PGE ₂	Prostaglandin E ₂
PGE ₃	Prostaglandin E ₃
PGH ₂	Prostaglandin H ₂
PGH ₃	Prostaglandin H ₃
PGJ ₂	Prostaglandin J ₂
PGJ ₃	Prostaglandin J ₃
PHA	Phytohaemagglutinin
PKC	Protein Kinase C
PMA	Phorbol Myristate Acetate
PP	Pulse Pressure
PPAR	Peroxisome Proliferator-Activated Receptor
PPL	Post-prandial Lipemia
QUICKI	Quantitative Insulin-Sensitivity Check Index
RCT	Randomised Controlled Trial
RER	Respiratory Exchange Ratio
ROS	Reactive Oxygen Species
s	second
SAC	Small Artery Compliance
SBP	Systolic Blood Pressure

SEM	Standard Error of Mean
SREBP-1c	Sterol Regulatory Element Binding Protein
SO	Sunflower Oil
SOX	Sunflower Oil + Exercise
SVR	Systemic Vascular Resistance
TAG	Triglyceride
TC	Total Cholesterol
TNF α	Tumor Necrosis Factor Alpha
TVI	Total Vascular Impedance
TX	Thromboxane
TXA ₂	Thromboxane A ₂
TXA ₃	Thromboxane A ₃
μ L	Microlitre
VCAM-1	Vascular Cell Adhesion Molecule-1
VLCD	Very Low Calorie Diet
VLDL	Very Low Density Lipoprotein
VO ₂	Oxygen Uptake
VCO ₂	Carbon Dioxide Output
WC	Waist Circumference
WHO	World Health Organisation
WHR	Waist to Hip Ratio
WLFO	Weight Loss with Fish Oil
WLPO	Weight Loss with Placebo Oil

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PUBLICATIONS ARISING FROM THIS THESIS

Papers – Published

AM Hill, J LaForgia, AM Coates, JD Buckley & PRC Howe. Estimating abdominal adipose tissue with dual energy x-ray absorptiometry and anthropometry. *Obesity*. 2007; 5: 504-510.

Buckley JD, **Hill AM**, Coates AM, Howe PRC. Simpler diet and exercise strategies for managing obesity in “Physical activity and obesity”. Editors Hills AP, Bryne NM and King NA. Smith-Gordon and Co, London (Published August 2006).

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Hill AM, Murphy KJ, Saint DA, Buckley JD, Howe P. Combined effects of omega-3 (ω 3) and moderate exercise on body fat and cardiovascular (CV) risk factors. *The Australian Society for Medical Research (SA Division) Annual Scientific Meeting*, 4th June 2004; abstr. O30.

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AM Hill, J LaForgia, AM Coates, JD Buckley & PRC Howe. DXA and anthropometry predict intra abdominal adipose tissue. *Physical Activity & Obesity. International Congress Satellite Conference*. September 2006, Brisbane, Australia.

AM Hill, JD Buckley, KJ Murphy & PRC Howe. Running on fish oil: benefits of ω 3 supplementation and exercise. *7th Congress for the International Society for the Study of Fatty Acids and Lipids*, July 2006, Cairns, Australia.

Hill AM, Buckley JD, Murphy KJ, Saint DA, Morris AM & Howe PRC Combined effects of omega-3 supplementation and regular exercise on body composition and cardiovascular risk factors. *Nutrition Society of Australia, 29th Annual Scientific Meeting*, December 2005, Melbourne, Australia.

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Poster Presentations

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