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The effect of exercise and diet on rat skeletal muscle phospholipid molecular species profile: an electrospray ionisation mass spectrometric analysis

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THE EFFECT OF EXERCISE AND DIET ON RAT SKELETAL MUSCLE PHOSPHOLIPID MOLECULAR SPECIES PROFILE: AN ELECTROSPRAY IONISATION MASS SPECTROMETRIC ANALYSIS

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Declaration

I, Todd W. Mitchell declare that this thesis, submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Biomedical Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Todd W Mitchell

6th October 2004

Dedication

This thesis is dedicated to my parents and my wife Tracy for their endless love and support and to my son Zane whose strength and determination to survive after a very early arrival has inspired me to realise my goals without losing sight of what is most important.

Abstract

Biological membranes separate cells from the external milieu and compartmentalise organelles within a cell, providing a specialised environment for many specific biochemical processes. They exist as bilayers of amphipathic lipids arranged with their hydrophobic moieties internalised and their hydrophilic regions directed to the membrane surfaces. Numerous proteins are also associated with membranes and are bound to the lipids by ionic or hydrophobic interactions. Phospholipids, however, are the major constituent of biological membranes and thus have a large influence upon the physical properties of the membrane and the many cellular functions membranes participate in.

To date our understanding of membrane lipid composition has been limited to phospholipid class or fatty acid analysis, primarily by thin layer chromatography, high performance liquid chromatography and gas chromatography. The results obtained by these techniques provide considerable evidence demonstrating an association between various metabolic disorders, such as insulin resistance and obesity and skeletal muscle phospholipid content. There is also a large pool of evidence confirming an effect of diet and exercise on the phospholipid fatty acid content of skeletal muscle membranes. Furthermore, these changes appear to have ameliorating effects upon the aforementioned metabolic disorders. An understanding of alterations in whole phospholipid molecular species induced by exercise and diet, however, is very limited. Recent advances in mass spectrometry allow the analysis of biological membranes at this whole molecule level.

In this thesis, a comparative analysis of skeletal muscle phospholipid molecular species profile between oxidative and glycolytic rat skeletal muscle and the effect of exercise and diet on these profiles have been performed using electrospray ionisation mass spectrometry (ESI-MS). Therefore, the primary aim of this thesis was to develop mass spectrometric techniques for analysing relative changes in phospholipid molecular species profile using a hybrid quadrupole time-of–flight (Q-ToF) mass spectrometer. To achieve this both total lipid and phospholipid extracts from various rat tissues such as brain, liver and skeletal muscle were obtained and used to (i) optimise instrument settings, (ii) ensure accurate identification of phospholipid molecular species, and (iii) ensure the

reproducibility of results. A normalisation procedure was then developed so that comparative analysis between groups could be performed. This was achieved by presenting the ion abundance of each phospholipid molecular species (after isotope corrections) as a percentage of the total ion abundance of all identified phospholipids within the *m/z* range analysed. The results obtained by the developed MS method were then compared to those attained by established GC methods and found to be in agreement, thus demonstrating the validity of the technique.

The methodology thus established was used to determine the effect of two exercise training intensities on the phospholipid profile of both glycolytic and oxidative muscle fibres of female Sprague-Dawley rats fed a standard laboratory chow diet. Animals were divided randomly into three training groups: control, which performed no exercise training; low intensity (8 m min^{-1}) treadmill running; or high intensity (28 m min^{-1}) treadmill running. All exercise-trained rats ran 1000 m $session^{-1}$, 4 days wk^{-1} for 4 wks and were killed 48 h after the last training bout. Exercise training was found to produce no novel phospholipid species but was associated with significant alterations in the relative abundance of a number of phospholipid molecular species. These changes were more prominent in glycolytic (white vastus lateralis) than in oxidative (red vastus lateralis) muscle fibres. The largest observed change was a decrease of approximately 20 % in the abundance of 1-stearoyl-2-docosahexaenoyl phosphatidylethanolamine [PE(18:0,22:6), $P<0.001$] ions in both the low and high intensity training regimes in glycolytic fibres. Increases in the abundance of 1-oleoyl-2-linoleoyl phopshatidic acid [PA(18:1,18:2), $P<0.001$] and 1-alkenylpalmitoyl-2-linoleoyl phosphatidylethanolamine [Plasmenyl PE(16:0,18:2), $P<0.005$] ions were also observed for both training regimes in glycolytic fibres.

The same exercise protocol was then performed by Sprague-Dawley rats fed a carbohydrate-free, high-fat diet and the skeletal muscle phospholipid molecular species profiles analysed. In agreement with the previous study, no novel molecular species were observed in the exercised rats yet significant changes in the relative abundance of various phospholipid molecular species were apparent. In contrast, however, the observed changes were more prominent in oxidative than glycolytic muscle fibres. The largest effect of exercise was found to be an increase of approximately 28 % in 1-

palmitoyl-2-linoleoyl phosphatidylcholine [PC(16:0,18:2), $P<0.05$] ions in oxidative muscle of rats in the low intensity training group when compared to the sedentary animals.

The phospholipid molecular species profile was found to be similar in both the oxidative and glycolytic muscles, however, a number of differences in the abundance of particular molecular species were observed. Of particular interest is the higher abundance of PE(18:0,22:6) in red vastus lateralis when compared to white vastus lateralis. In spite of the fact that the high-fat diet was completely deficient in n-3 polyunsaturated fatty acids the ratio of PE(18:0,22:6) in oxidative to glycolytic muscle was almost identical across both diet groups. For example, in the sedentary rats this ratio was 1.35 for the carbohydrate diet group and 1.32 for the fat diet group.

It is concluded that exercise training results in a significant level of membrane remodelling at the level of phospholipid molecular species and that traditional methods used to analyse phospholipids such as TLC and GC are not able to uncover these changes. Moreover, it is probable that the observed changes will have effects upon the activity of various membrane bound proteins and in turn cell function. At present an understanding of the role specific phospholipid molecular species play in membrane function is extremely limited and further correlative and manipulative studies are required to remedy this. It is likely that electrospray ionisation mass spectrometry will play a significant role in these future studies.

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ABBREVIATIONS

ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionisation
BMR	Basal metabolic rate
CAD	Collision-activated dissociation
CI	Chemical ionisation
CID	Collision-induced dissociation
CRM	Charge residue model
DEFA	Deficient in essential fatty acids
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DPG	Diphosphatidylglycerol (Cardiolipin)
ED_{50}	Half-maximal glucose disposal
EDL	Extensor digitorum longus
EI	Electron ionisation
EPA	Eicosapentaenoic acid
ESI	Electrospray ionisation
ESI-MS	Electrospray ionisation mass spectrometry
FA	Fatty acid
FAB	Fast atom bombardment
GC	Gas chromatography
GLUT	Glucose transporter
HIGH	High intensity exercise training
HPLC	High performance liquid chromatography
IEM	Ion evaporation model
LC/MS	Liquid chromatography mass spectrometry
LOW	Low intensity exercise training
LSIMS	Liquid secondary ion mass spectrometry
m/z	Mass to charge ratio
MALDI	Matrix-assisted laser desorption/ionisation
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MUFA	Monounsaturated fatty acid
oaToF	Orthogonal acceleration time-of-flight
PA	Phosphatic acid

PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PLA_1	Phospholipase A_1
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PLD	Phospholipase D
PS	Phosphatidylserine
PSD	Post source decay
PUFA	Polyunsaturated fatty acid
Q-ToF	Quadrupole time-of-flight
RF	Radio frequency
RVL	Red vastus lateralis
SED	Sedentary
SEM	Standard error of the mean
SFA	Saturated fatty acid
SIMS	Secondary ion mass spectrometry
SM	Sphingomyelin
TAG	Triacylglycerol
TLC	Thin layer chromatography
ToF	Time-of-flight
VO_{2max}	Maximal oxygen uptake
WVL	White vastus lateralis

PUBLICATIONS ARISING FROM WORK IN THIS THESIS TO DATE

Journal Articles

Turner N, Lee JS, Bruce CR, **Mitchell TW**, Else PL, Hulbert AJ & Hawley JA (2004) Greater effect of diet than exercise training on the fatty acid profile of rat skeletal muscle. *Journal of Applied Physiology* 96, 974-980.

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