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Dietary omega-3 fatty acids, nutritional preconditioning of the heart against ischemic injury

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DIETARY OMEGA-3 FATTY ACIDS, NUTRITIONAL PRECONDITIONING OF THE HEART AGAINST ISCHEMIC INJURY

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CERTIFICATION

I, Grace Gulbahar Abdukeyum, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the school of health 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.

Grace Gulbahar Abdukeyum

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GLOSSARY OF TERMINOLOGY

Acute coronary occlusion: The partial or complete obstruction of blood flow in a coronary artery.

Afterload: Resistance to ventricular ejection: measured clinically with aortic blood pressure and calculation of systemic vascular resistance.

Angina: Chest pain or discomfort that occurs when an area of heart muscle does not get enough oxygen-rich blood.

Apoptosis: Occurrence of internucleosomal fragmentation of genomic DNA associated with a sealed plasma membrane.

Bradyarrhythmias: Slow heart rate, an arrhythmia caused by failure of impulse formation or by failure of impulse conduction.

Cardiac arrest: The sudden complete loss of cardiac output and therefore blood pressure.

Cardiac Output: Cardiac output is the volume of blood being pumped by the heart, in particular by a ventricle in a minute. Cardiac output is equal to the stroke volume multiplied by the heart rate .

Cardiac arrhythmia (also dysrhythmia): Abnormal electrical activity in the heart. The heartbeat may be fast or slow, and may be regular or irregular.

Coronary Artery Bypass Graft Surgery: Colloquially heart bypass or bypass surgery is a surgical procedure where arteries or veins from elsewhere in the patient's body are grafted to the coronary arteries to bypass atherosclerotic

narrowing and improve the blood supply to the coronary circulation supplying the myocardium.

Cost-effectiveness ratios: A ratio of the change in costs of a therapeutic intervention to the change in effects of the intervention.

Diastolic arterial pressure: The lowest pressure (at the resting phase of the cardiac cycle). Typical values for a resting, healthy adult human are approximately 120mmHg systolic and 80mmHg diastolic with large individual variations.

End-diastolic pressure: The pressure in the ventricles at the end of diastole, usually measured in the left ventricle as an approximation of the end-diastolic volume, or preload.

End-diastolic volume: The volume of blood in a ventricle at the end of filling (diastole). Because greater end diastolic volumes cause greater distention of the ventricle, end diastolic volume is often used synonymously with preload.

Fibrin: Insoluble protein that is produced in response to bleeding and is the major component of the blood clot.

Free radicals: Any atomic or molecular species capable of independent existence that contains one or more unpaired electrons in one of its molecular orbitals.

Ischemia: Oxygen deprivation accompanied by inadequate removal of metabolites due to reduced perfusion. Prolonged ischemia results in tissue damage and death because of a lack of oxygen and nutrients.

Hemostasis: Refers to the physiologic process whereby bleeding is halted, thus protecting the integrity of the vascular system after tissue injury.

Ischemia reperfusion: The restoration of blood flow to an area that had previously experienced deficient blood flow.

Ischemic heart disease: A condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium; it typically occurs when there is an imbalance between myocardial oxygen supply and demand.

Ischemic reperfusion injury: Accelerated and additional myocardial injury beyond that generated by ischemia alone. It includes hastening of the necrotic process of irreversibly injured myocytes, cell swelling, the no-reflow phenomenon, hemorrhagic myocardial infarction, the calcium and oxygen paradox, the production of oxygen-derive free radicals which may damage ischemic myocytes, and the prolonged postischemic depression of ventricular function and electrophysiologic changes which in turn can cause arrhythmias.

Ischemic preconditioning: A technique in which tissue is rendered resistant to the deleterious effects of prolonged ischemia and reperfusion by prior exposure to brief, repeated periods of vascular occlusion.

Myocardial infarction (MI): A loss of cardiac myocytes (necrosis) caused by prolonged ischemia.

Myocardial stunning: The mechanical dysfunction that persists after reperfusion, despite the absence of irreversible damage and restoration of normal coronary flow.

Necrosis: The ultimate form of cell death and defined as accidental collapse of cellular homeostasis, compartmentalisation and cell membrane integrity with the release of cytosolic material and with random nuclear DNA fragmentation.

Nutritional preconditioning: Inducing preconditioning by nutritional means.

(n−3) fatty acids: n−3 fatty acids (popularly referred to as omega-3 fatty acids or ω−3 fatty acids) are a family of unsaturated fatty acids that have in common their

first carbon–carbon double bond as the third bond from the methyl end of the fatty acid.

(n−6) fatty acids: n−6 fatty acids (popularly referred to as omega-6 fatty acids or ω−6 fatty acids) are a family of unsaturated fatty acids which have in common their first carbon–carbon double bond as the sixth bond from the methyl end of the fatty acid.

Oncosis: Early plasma membrane rupture and disruption of cellular organelles, including mitochondria.

Percutaneous transluminal coronary angioplasty: Commonly known as coronary angioplasty or simply angioplasty, a form of catheter-based therapy as an alternative to bypass surgery is to treat the stenotic coronary arteries of the heart found in coronary heart disease. Angioplasty is less invasive than coronary artery bypass surgery.

Plasminogen: The inactive precursor of plasmin, a potent serine protease involved in the dissolution of fibrin blood clots.

Primary prevention: Can include prevention of atherosclerosis, acute coronary occlusion myocardial infarction and sudden cardiac death.

Pharmacological preconditioning: Inducing preconditioning by pharmacological means.

Preload: The pressure stretching the ventricular wall of the heart after passive filling and atrial contraction. Preload is theoretically most accurately described as the initial stretching of a single cardiac myocyte prior to contraction.

Secondary prevention: Prevention of fatal arrhythmias or new ischemic episodes in those who have had a prior heart attack.

Stroke volume: The volume of blood pumped out of one ventricle of the heart in a single beat.

Stroke work: The work done by the ventricle to eject a volume of blood (i.e., stroke volume) into the aorta.

Stunning: Viable myocardium, which is not contracting properly but is not acutely ischemic.

Systolic arterial pressure: The peak pressure in the arteries, which occurs near the beginning of the cardiac cycle.

Sudden cardiac death: Natural death from cardiac causes, heralded by abrupt loss of consciousness within one hour of the onset of acute symptoms; preexisting heart symptoms may have been known to be present, but the time and mode of death are unexpected.

Thrombus or **blood clot**: The final product of the blood coagulation step in hemostasis.

Thrombosis: The formation of a clot or thrombus inside a blood vessel, which obstructs the flow of blood through the circulatory system.

Ventricular tachycardia: Three or more consecutive ventricular premature beats. If persistent, causes loss of blood pressure and death.

Ventricular fibrillation: A rapid, chaotic, and asynchronous contraction of the left ventricle.

ABSTRACT

Cardiovascular disease now ranks as the leading cause of death, resulting in one third of all deaths in the world, among which ischemic heart disease is projected to be the number one cause of death globally. It has been long suggested that (n-3) polyunsaturated fatty acids provide cardiovascular protection, with regular intake of fish or fish oil associated with reduced mortality from heart diseases in both clinical trials and epidemiological studies. One major observation in these studies is that fish oil has been found to reduce mortality during or following ischemic events. While ischemia damages the heart, short bursts of ischemia paradoxically protect the heart from the damaging effects of more prolonged ischemia. This powerful cardioprotective phenomenon is termed ischemic preconditioning. The protective influences of ischemic preconditioning include reduction of infarct size, prevention of life-threatening arrhythmias in ischemia and reperfusion, reduced myocardial oxygen demand, and improved recovery of post-ischemic cardiac pumping function. However, the promise of ischemic preconditioning has not yet been realized in bench to bedside application. The characteristics of cardioprotection afforded by feeding (n-3) PUFA suggests preconditioning-like effects, related to incorporation of (n-3) PUFA into heart membranes. However, the long chain highly unsaturated (n-3) PUFA in the membranes are highly susceptible to peroxidation, perhaps making cardiac membranes more susceptible to free radical generation and cellular damage. While free radicals are thought to be involved in the damaging

effects of ischemia and reperfusion, they also play a role in protective mechanisms of ischemic preconditioning.

This study evaluated the effects of fish oil on (n-3) PUFA incorporation into myocardial membrane and examined the susceptibility to oxidative damage and myocardial injury in terms of infarct size and postischemic cardiac function. Further, it compared dietary fish oil with ischemic preconditioning and assessed their interaction for effects on heart function and injury during myocardial ischemia and reperfusion. It tested the hypothesis that the susceptibility to peroxidation may provide an ever-present preconditioning stimulus that protects the heart against the damaging effects of a major ischemia reperfusion insult.

Male Wistar rats were fed one of three fully fabricated diets containing 10% fat by weight varying only in the types of fat. The (n-3) PUFA diet contained 7% fish oil + 3% olive oil; The (n-6) PUFA diet contained 5% sunflower seed oil + 5% olive oil; The saturated fatty acid (SF) diet contained 7% saturated fat-rich beef tallow + 3% olive oil. Heart function was examined after six weeks feeding using Langendorff-perfused isolated isovolumic heart preparation. In control experiments, isolated perfused hearts were subjected to 30 minutes regional ischemia by occluding the left anterior descending coronary artery, then reperfused for 120 minutes. Ischemic preconditioning consisted of three cycles of five minutes global ischemia before the 30 minutes regional ischemia and 120 minutes reperfusion. Heart function was assessed during perfusion by ECG and by measurement of intraventricular pressure. Infarct size was measured at completion

of perfusion in control and ischemic preconditioned hearts as a percent of the ischemic zone at risk. Lipid peroxidation products and antioxidant concentrations were measured in normoxic heart and in ischemic and non-ischemic regions of hearts with or without ischemic preconditioning.

Control (n-3) PUFA hearts had significantly lower spontaneous heart rate, coronary flow, end diastolic pressure, maximum relaxation rate, and fewer ischemic reperfusion arrhythmias than did (n-6) PUFA hearts or SF hearts. In reperfusion (n-3) PUFA hearts maintained greater developed pressure and maximum rate of relaxation and developed smaller infarcts (10.9±3.6% ischemic zone, n=6) than (n-6) PUFA hearts (47.4±2.3%, n=6) or SF hearts (50.3±4.3%, n=6).

Ischemic preconditioning significantly improved heart function and reduced infarct size in (n-6) PUFA hearts (11.8±5.4%, n=6) and SF hearts (13.1±4.2%, n=6). Heart function and infarct size did not differ between control and ischemic preconditioned hearts (9.6±4.2%) with (n-3) PUFA diet. Arrhythmias were significantly reduced by ischemic preconditioning in (n-6) PUFA hearts or saturated fatty acid hearts towards levels observed in (n-3) PUFA hearts.

Myocardial membranes showed high incorporation of long chain docosahexaenoic acid (DHA) (22:6,n-3), predicting increased risk of peroxidation. The concentration of lipid hydroperoxides and malondialdehyde were higher in normoxic and nonischemic regions of control (n-3) PUFA hearts than in (n-6) PUFA or SF hearts. The concentration of the endogenous antioxidant superoxide dismutase was higher

in normoxic and nonischemic regions of control (n-3) PUFA hearts and was increased after ischemic preconditioning in saturated fatty acid and (n-6) PUFA hearts. Both (n-3) PUFA diet and ischemic preconditioning inhibited the ischemia-induced rise in the oxidation products lipid hydroperoxides and malondialdehyde.

This thesis demonstrated that the harmful effects of myocardial ischemia and reperfusion, such as infarct size, poor relaxation, cardiac arrhythmia and poor recovery of contractile function were largely curtailed by feeding an (n-3) PUFA rich diet. The effects of SF and (n-6) PUFA diet on heart function and ischemia susceptibility were largely indistinguishable, indicating that the effects of fish oil were specifically related to its (n-3) PUFA content and not due to either a reduction in saturated fat intake or a non-specific role of polyunsaturated fatty acids. Regular consumption of dietary fish oil induced sustained changes in membrane fatty acid composition and produced cardioprotection that appears similar to late ischemic preconditioning. The continuous presence of (n-3) PUFA in myocardial membranes suggests that, as reported for late ischemic preconditioning, this is not subject to desensitization.

Dietary treatment of rats with (n-3) PUFA caused an increase in peroxidation index suggesting an increase in susceptibility of the membrane to oxidative damage, which might be expected to enhance ischemic damage. An increase in membrane lipid peroxidation was indeed observed in fish oil treated rat hearts, however, it was associated with increased antioxidant activity and reduced lipid oxidation under stress and instead of causing lasting damage to heart function, beneficial effects on

arrhythmia, contractile function, and myocardial infarct size were observed. These protective effects are demonstrated as powerful as ischemic preconditioning.

Therefore, in light of cardioprotective effects of (n-3) PUFA to reduce the consequences of ischemic events in the human population when a regular part of the diet, the present thesis demonstrated that (n-3) PUFA induces a form of preconditioning in the heart, which this thesis has termed, nutritional preconditioning. The (n-3) PUFA limit ischemic cardiac injury and myocardial infarction and endow cardioprotection as powerful as ischemic preconditioning under these experimental conditions. Nutritional preconditioning by membrane incorporation of (n-3) PUFA may underpin the low cardiovascular morbidity and mortality associated with regular fish and fish oil consumption.

PUBLICATION ARISING FROM THIS THESIS

Abdukeyum, G.G., Owen, A.J., and McLennan, P.L. (2008), 'Dietary (n-3) long-chain polyunsaturated fatty acids inhibit ischemia and reperfusion arrhythmias and infarction in rat heart not enhanced by ischemic preconditioning', *J. Nutr.,* 138 (10), 1902-09.

Abdukeyum, G.G., Owen, A.J., and McLennan, P.L. (2008), 'Effect of ischemic preconditioning and fish oil on oxidative status in isolated rat hearts' (Pending submission for publication*).*

PUBLISHED ABSTRACTS PRESENTED AT INTERNATIONAL MEETINGS

Abdukeyum, G.G. and McLennan, P.L. (2007), 'Dietary fish oil mimics the cardio-protective effect of ischemic preconditioning in isolated rat heart', *FASEB J.,* 21 (6), A1223-c-.

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PUBLICATION FROM OTHER WORK DURING CANDIDATURE

Dietrich, M., Hu, Y.Q., Block, G., Olano, E., Packer, L., Morrow, J.D., Hudes, M., Abdukeyum, G.G., et al. (2005), 'Associations between apolipoprotein e genotype and circulating F-2-isoprostane levels in humans', *Lipids,* 40 (4), 329-34.