Chapter 1. Introduction

This evidence report is one of three reports prepared by the Tufts-New England Medical Center (Tufts-NEMC) Evidence-based Practice Center (EPC) concerning the health benefits of omega-3 fatty acids on cardiovascular diseases. These reports are among several that address topics related to omega-3 fatty acids, and that were requested and funded by the Office of Dietary Supplements, National Institutes of Health (NIH) through the EPC program at the Agency for Healthcare Research and Quality (AHRQ). Three EPCs — the Tufts-NEMC EPC, the Southern California-RAND EPC, and the University of Ottawa EPC — each produced evidence reports. To ensure consistency of approach, the three EPCs collaborated on selected methodological elements, including literature search strategies, rating of evidence, and data table design.

The aim of these three reports is to summarize the current evidence on the health effects of omega-3 fatty acids on the following: cardiovascular diseases, cancer, child and maternal health, eye health, gastrointestinal/renal diseases, asthma, autoimmune diseases, immune-mediated diseases, transplantation, mental health, and neurological diseases and conditions. In addition to informing the research community and the public on the effects of omega-3 fatty acids on various health conditions, it is anticipated that the findings of the reports will also be used to help define the agenda for future research.

The focus of this report is on the effect of omega-3 fatty acids on cardiac electrogenesis and arrhythmias. The other two reports focus on the effects of omega-3 fatty acids on cardiovascular disease and effects of omega-3 fatty acids on cardiovascular disease risk factors. In this chapter, we review the metabolism, physiological functions, and sources of omega-3 fatty acids. In addition, we examine some basic aspects of cardiac electrophysiology or electrogenesis and discuss the analytic framework for this report. Subsequent chapters describe the methods used to identify and review studies related to omega-3 fatty acids and cardiac electrogenesis, findings related to the effects of omega-3 fatty acids on cardiac electrogenesis and arrhythmias, and recommendations for future research in this area.

Metabolism and Biological Effects of Essential Fatty Acids

Dietary fat is an important source of energy for biological activities in human beings. Dietary fat encompasses saturated fatty acids, which are usually solid at room temperature, and unsaturated fatty acids, which are liquid at room temperature. Unsaturated fatty acids can be further divided into monounsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) can be classified on the basis of their chemical structure into two groups: omega-3 (n-3) fatty acids and omega-6 (n-6) fatty acids. The *omega-3* or *n-3* notation means that the first double bond from the methyl end of the molecule is in the third. The same principle applies to the *omega-6* or *n-6* notation. Despite their differences in structure, all fats contain the same amount of energy (9 kcal/g or 37 kJ/g).

Of all fats found in food, 2 — alpha-linolenic acid (chemical abbreviation: ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) — cannot be synthesized in the human body, yet are necessary for proper physiological functioning. These 2 fats are called essential fatty acids. The essential fatty acids can be converted in the liver to long-chain polyunsaturated fatty acids (LC PUFAs), which

have a higher number of carbon atoms and double bonds. These LC PUFAs retain the omega type (n-3 or n-6) of the parent essential fatty acids.

ALA and LA comprise the bulk of the total PUFAs consumed in a typical North American diet. Typically, LA comprises 89% of the total PUFAs consumed, while ALA comprises 9%. Smaller amounts of other PUFAs make up the remainder [1]. Both ALA and LA are present in a variety of foods. For example, LA is present in high concentrations in many commonly used oils, including safflower, sunflower, soy, and corn oil. ALA, which is consumed in smaller quantities, is present in leafy green vegetables and in some commonly used oils, including canola and soybean oil. Some novelty oils, such as flaxseed oil, contain relatively high concentrations of ALA, but these oils are not commonly found in the food supply.

The Institute of Medicine suggests that, for adults 19 and older, an adequate intake (AI) of ALA is 1.1-1.6 g/day, while an adequate daily intake of LA is 11-17 g/day [2]. Recommendations regarding AI differ by age and gender groups, and for special conditions such as pregnancy and lactation.

As shown in Figure 1.1, EPA and DHA can act as competitors for the same metabolic pathways as AA. In human studies, the analyses of fatty-acid compositions in both blood phospholipids and adipose tissue showed similar competitive relationship between omega-3 LC PUFAs and AA. General scientific agreement supports an increased consumption of omega-3 fatty acids and reduced intake of omega-6 fatty acids to promote good health. However, for omega-3 fatty acid intakes, the specific quantitative recommendations vary widely among countries not only in terms of different units — ratio, grams, total energy intake — but also in quantity [3]. Furthermore, there remain numerous questions relating to the inherent complexities about omega-3 and omega-6 fatty acid metabolism, in particular regarding the inter-relationships between the 2 fatty acids. For example, it remains unclear to what extend ALA is converted to EPA and DHA in humans, and to what extend high intake of omega-6 fatty acids compromises any benefits of omega-3 fatty acid consumption. Without resolution of these 2 foundational questions, it remains difficult to study the importance of omega-6 to omega-3 fatty acid ratio.

Metabolic Pathways of Omega-3 and Omega-6 Fatty Acids

Omega-3 and omega-6 fatty acids share the same pools of enzymes and go through the same oxidation pathways while being metabolized (Figure 1.1). Once ingested, ALA and LA can be elongated and desaturated into LC PUFAs. LA is converted into gamma-linolenic acid (GLA, 18:3 n-6), an omega-6 fatty acid that is a positional isomer of ALA. GLA, in turn, can be converted to the long-chain omega-6 fatty acid, arachidonic acid (AA, 20:4 n-6). ALA can be converted, to a lesser extent, to the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). However, the conversion from parent fatty acids into LC PUFAs occurs slowly in humans, and conversion rates are not well understood. Because of the slow rate of conversion and the importance of LC PUFAs to many physiological processes, humans must augment their level of LC PUFAs by consuming foods that are rich in these important compounds. Meat is the primary food source of AA, while fish is the primary food source of EPA.

The specific biological functions of fatty acids depend on the number and position of double bonds and the length of the acyl chain. Both EPA and AA are 20-carbon fatty acids and are precursors for the formation of prostaglandins, thromboxane, and leukotrienes — hormone-like

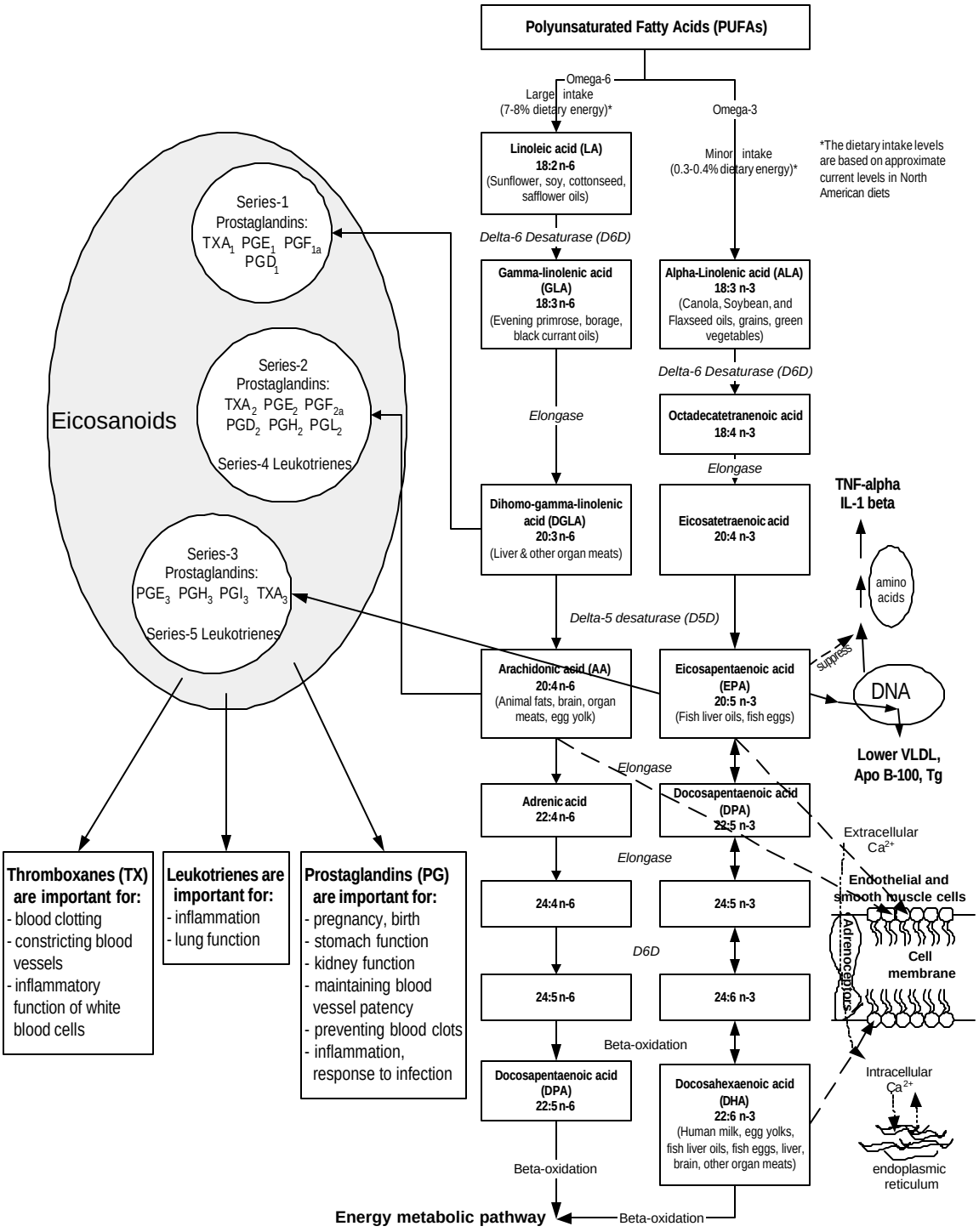
agents that are members of a larger family of substances called eicosanoids. Eicosanoids are localized tissue hormones that seem to be one of the fundamental regulatory classes of molecules in most higher forms of life. They do not travel in the blood, but are created in the cells to regulate a large number of processes, including the movement of calcium and other substances into and out of cells, dilation and contraction of muscles, inhibition and promotion of clotting, regulation of secretions including digestive juices and hormones, and control of fertility, cell division, and growth [4].

As shown in Figure 1.1, the long-chain omega-6 fatty acid, AA, is the precursor of a group of eicosanoids including series-2 prostaglandins and series-4 leukotrienes. The omega-3 fatty acid, EPA, is the precursor to a group of eicosanoids including series-3 prostaglandins and series-5 leukotrienes. The series-2 prostaglandins and series-4 leukotrienes derived from AA are involved in intense actions (such as accelerating platelet aggregation and enhancing vasoconstriction and the synthesis of inflammatory mediators) in response to physiological stressors. The series-3 prostaglandins and series-5 leukotrienes that are derived from EPA are less physiologically potent than those derived from AA. More specifically, the series-3 prostaglandins are formed at a slower rate and work to attenuate excessive series-2 prostaglandins. Thus, adequate production of the series-3 prostaglandins, which are derived from the omega-3 fatty acid, EPA, may protect against heart attack and stroke as well as certain inflammatory diseases like arthritis, lupus, and asthma [4]. In addition, animal studies, have demonstrated that omega-3 LC PUFAs, such as EPA and DHA, engage in multiple cytoprotective activities that may contribute to antiarrhythmic mechanisms[5]. Arrhythmias are a common cause of “sudden death” in heart disease.

In addition to affecting eicosanoid production as described above, EPA also affects lipoprotein metabolism and decreases the production of other compounds — including cytokines, interleukin 1ß (IL-1ß), and tumor necrosis factor a (TNF-a) — that have pro-inflammatory effects. These compounds exert pro-inflammatory cellular actions that include stimulating the production of collagenases and increasing the expression of adhesion molecules necessary for leukocyte extravasation [6]. The mechanism responsible for the suppression of cytokine production by omega-3 LC PUFAs remains unknown, although suppression of eicosanoid production by omega-3 fatty acids may be involved. EPA can also be converted into the longer chain omega-3 form of docosapentaenoic acid (DPA, 22:5 n-3), and then further elongated and oxygenated into DHA. EPA and DHA are frequently referred to as very long chain omega-3 fatty acids. DHA, which is thought to be important for brain development and functioning, is present in significant amounts in a variety of food products, including fish, fish liver oils, fish eggs, and organ meats. Similarly, AA can convert into an omega-6 form of DPA. Studies have reported that omega-3 fatty acids decrease triglycerides (Tg) and very low density lipoprotein (VLDL) in hypertriglyceridemic subjects, with a concomitant increase in high density lipoprotein (HDL). However, they appear to increase or have no effect on low density lipoprotein (LDL). Omega-3 fatty acids apparently lower Tg by inhibiting VLDL and apolipoprotein B-100 synthesis and decreasing post-prandial lipemia [7]. Omega-3 fatty acids, in conjunction with transcription factors (small proteins that bind to the regulatory domains of genes), target the genes governing cellular Tg production and those activating oxidation of excess fatty acids in the liver. Inhibition of fatty acid synthesis and increased fatty acid catabolism reduce the amount of substrate available for Tg production [8]

As noted earlier, omega-6 fatty acids are consumed in larger quantities (>10 times) than omega-3 fatty acids. Maintaining a sufficient intake of omega-3 fatty acids is particularly important since many of the body’s physiologic properties depend upon their availability and metabolism.

Figure 1.1. Classical omega-3 and omega-6 fatty acid synthesis pathways and the role of omega-3 fatty acid in regulating health/disease markers.



Overview of the Electrophysiology of the Heart

In this report, we examine evidence that omega-3 fatty acids affect cell organelles — such as cardiac ion channels, pumps, or exchange mechanisms — that are involved in cardiac electrophysiology or electrogenesis. This section of the report reviews some basic aspects of electrogenesis and omega-3 fatty acids., and discusses the analytic framework that guided our systematic review of the literature. Two accompanying reports --- *Effects of Omega-3 Fatty Acids on Cardiovascular Disease Risk Factors* and *Effects of Omega-3 Fatty Acids on Cardiovascular Disease* review evidence from clinical studies focused on the relationship between omega-3 fatty acids and outcomes in humans including sudden death.

Cardiac Electrophysiology

The heart's beating rate is controlled by specialized, spontaneously firing pacemaker cells in the sino-atrial node (a bundle of specialized cardiac muscle cells in the right atrium of the heart) and by sympathetic and parasympathetic nerve fibers that influence the ion balance in heart muscle cells. The pacemaker cells initiate an electrical impulse that produces a change in the voltage of heart cell membranes. This change in voltage, also called an action potential, is generated by the relative concentration of different types of ions across the cell membrane, and moves from one heart muscle cell (or myocyte) to another [9].

Calcium, potassium, and sodium ions are central to the generation of action potentials. These ions, in the form of currents, move across cell membranes through pathways called channels. The speed at which ions traverse these channels varies due to channel characteristics. Some channels open or close as a function of membrane potential, while others respond to neurotransmitters or other molecules. Sodium and calcium ions also use an energy-dependent pumping process to cross the membrane. [9].

These electrophysiological processes interact with structural components of cardiac myocytes to cause synchronized contraction and relaxation of the heart muscle. The sarcoplasmic reticulum (SR) — a system of membranes in cardiac muscle cells — stores calcium ions during the diastolic, or relaxation, phase of the contraction cycle. Infoldings of the cell membrane (or sarcolemma) called T-tubules transmit the action potential along the membrane far into the cell. The resulting excitation-contraction coupling process increases the concentration of intracellular free calcium ions during depolarization across the cell membrane and T-tubules. The calcium ions facilitate muscle contraction by interacting with other cellular components. The exchangers and pumps that support the contractile process rely on the presence of adenosine triphosphate (ATP) and are affected by the concentration gradients of sodium, potassium, and calcium ions. The strength of cardiac muscle contraction, or myocardial contractility, can be increased by norepinephrine, which is secreted by sympathetic nerves and mediated by ß-adrenergic receptors and calcium channels. Myocardial muscle relaxation occurs when the calcium is returned to the sarcoplasmic reticulum or is pumped out of the cell by sodium-calcium exchangers and calcium adenosine triphosphatase (ATPase) pumps [9].

Arrhythmia

Cardiac arrhythmias, or disorders of the heart's rhythm, are a serious cause of morbidity and mortality. Serious arrhythmias can cause sudden death (abrupt loss of heart function or cardiac arrest) — a leading cause of death in industrialized societies. According to the Heart and Stroke Statistical Update for 2003 [10] arrhythmias were a direct cause of 37,646 deaths in the United States and were an underlying or contributing cause of another 491,000 deaths. In addition to contributing to sudden death, serious arrhythmias can compromise the normal flow of blood through the coronary arteries, resulting in impaired oxygenation of the heart muscle (myocardial ischemia) or death of cardiac muscle tissue (myocardial infarction or heart attack). Arrhythmias can also lead to other cardiovascular conditions, such as stroke, congestive heart failure, and peripheral embolism.

There are many potential causes of arrhythmias, including disruption of ion channels or pumps, reduction in blood flow to the heart muscle (ischemia), and alteration of the eicosanoid system and adrenoceptors (membrane proteins whose function in the heart is to transmit the neuroendocrine message sent by catecholamines like adrenaline and its derivatives). Changes in these systems result in electrical abnormalities in the heart leading to disturbances in the heart rhythm such as tachycardia, bradycardia, or uncoordinated contraction of the heart muscle cells.

A key purpose of this report is to examine the evidence that omega-3 fatty acids directly affect cell organelles such as cardiac ion channels, pumps, or exchange mechanisms involved in electrogenesis. The accompanying reports, entitled *Effects of Omega-3 Fatty Acids on Cardiovascular Disease Risk Factors* and *Effects of Omega-3 Fatty Acids on Cardiovascular Disease,* provide a review of the evidence from clinical studies of the effect of omega-3 fatty acids on arrhythmia and sudden death in humans.

Potential Impact of Omega-3 Fatty Acids on Arrhythmogenesis

As described above, cell organelles such as cardiac ion channels, pumps, currents, and exchange mechanisms are essential electrophysiological processes that ensure normal heart rate and coronary blood flow. These processes depend upon the concentration gradient of sodium, potassium, and calcium, and associated enzymes. Disruptions in these concentrations can lead to asynchronous contractility of the myocardium and result in arrhythmias. Clinically, the main causes of arrhythmia are ischemia, electrolyte disturbances, drugs, and underlying structural problems (e.g. bypass tracts). The physiologic mechanisms underlying these effects involve such mechanisms as ion channels and pumps and membrane currents.

Omega-3 LC PUFAs may exert an anti-arrhythmic effect on cardiac cells in several ways. For example, they can affect the adrenoceptors that transmit neuroendocrine messages sent by catecholamines. The omega-3 fatty acid, DHA, for instance, causes both a decrease in the production of cyclic adenosine monophosphate (cAMP), the main ß-adrenergic messenger, and an increase in chronotropic response or heart rate[11]. Omega-3 LC PUFAs also appear to act like another group of cardiovascular drugs, calcium channel blockers, by increasing intracellular calcium sequestration and interfering with receptor-operated calcium channels, influx [12].