Chapter 3. Results

In this chapter, we provide an overview of our literature search and discuss findings from the studies that met our search criteria. An overview of the literature search is presented first, followed by a review of whole animal studies, whole animal isolated organ and cell studies, and isolated organ and cell culture studies.

Literature Search Overview

Through the literature search, we identified 1,807 abstracts that met our search criteria. After screening the abstracts, we retrieved 274 articles. Of these, 184 were rejected after reviewing the full text. The reasons for rejection are as follows: no omega-3 fatty acids (30), not specific to arrhythmia (31), no cardiac cells (4), fatty acid composition or products only (34), other reasons (90). Details associated with the reasons for rejection are summarized in the reasons for rejection section. At the end of this process, 89 articles were accepted and reviewed.

For each class of study — whole animal studies, whole animal isolated organ and cell culture studies, and isolated organ and cell culture studies — we tabulated the outcomes/parameters measured by each investigator. Tables 3-1 to 3-3 summarize these parameters by species model and parameter.

Whole Animal Studies

A total of 26 whole animal studies (Tables 3-4 through 3-20 and Evidence Table 1) were reviewed. In 23 of the studies, omega-3 fatty acid supplements were added to the animals' food for a variable duration of time before experimental protocols for induced arrhythmias were implemented. In the remaining 3 studies, fatty acids were infused intravenously as a treatment to prevent induced or spontaneous arrhythmias. In the pre-fed route, dietary fatty acids must be incorporated into an animal's cell membrane before they can influence cell function and/or rehabilitation. In contrast, when omega-3 fatty acids are directly injected into an animal's blood stream, they exist and function in free form. The results of these two types of studies will be discussed separately, since their presumed physiological mechanisms differ. A summary of the 26 whole-animal studies is shown in Table 3-1.

Individual summary tables were created to show the effects of omega-3 fatty acids on various arrhythmic outcomes. Studies were grouped first by outcomes, then by species, and finally by experimental protocols (or mechanisms) for induced arrhythmias. Within each table, comparisons were first clustered into alpha linolenic acid (ALA, 18:3 n 3) oils or fish oils, and then sorted by the dose of omega-3 fatty acids. Frequently, studies had more than one comparison and used more than one experimental protocol. As a result, some studies appear multiple times in one table (once for each comparison group) or appear in several different tables.

In general, the arrhythmic outcomes assessed were defined consistently across the 26 whole-animal studies with the exception of the definition for arrhythmia score, which varied

somewhat across studies. The following arrhythmic outcome measures and general definitions were used in the original studies:

- **Ventricular Tachycardia (VT)**: A run of four or more consecutive ventricular premature beats [14].

- **Ventricular Fibrillation (VF)**: A signal for which individual QRS deflections can no longer be distinguished from one another (implying morphological instability) and for which a rate can no longer be measured [14].

- **Ventricular Premature Beats (VPB)**: Isolated ventricular premature beats are generally defined as discrete and identifiable premature QRS complexes (premature in relation to the P wave) [14].

- **Arrhythmia Score (AS)**: A hierarchical scale of 0 to 9 during occlusion as most described by Curtis et al., 1987 [15], and during reperfusion using a slightly modified version of the scale as described by McLennan et al., 1988 [16].

- **Infarct Size (IS)**: The under-perfused ischemic regions determined by dye exclusion and expressed as a percentage of wet weight in both ventricles [16]. In the studies examined for this report, infarct size reflects myocardial tissue that has sustained damage due to the ischemia procedures that were used to induce arrhythmias.

We performed meta-analyses for each of the outcomes. In these analyses, fish oils and ALA oils were analyzed separately and in combination.

In the following sections, the 23 pre-fed studies are discussed first and are grouped according to the comparison substance. Studies comparing omega-3 polyunsaturated fatty acids (PUFAs) to omega-6 PUFAs are presented first, followed by studies comparing omega-3 PUFAs to a-linolenic acid, monounsaturated fatty acids (MUFAs), saturated fatty acids (SFAs), and no treatment. The 3 studies that infused free form omega-3 fatty acids are reviewed at the end of the *Whole Animal Studies* section.

Studies Comparing Pre-Fed Omega-3 PUFAs to Omega-6 PUFAs

This section summarizes 13 studies that compared pre-fed omega-3 PUFAs to pre-fed omega-6 PUFAs (see Table 3-1 and Evidence Table 1). In each study, the same amount of experimental and control oil was added to each animal's basic diet. Therefore, all comparisons have iso-caloric intake from fat. The dose of omega-3 fatty acids ranged from 0.4 to 3.7g/100g. Fish oils (menhaden, tuna fish oils, or MaxEPA---a commercial preparation of EPA), soybean oil, or canola oil were used as the source of omega-3 PUFAs in the experimental groups, while controls were fed sunflower seed oil, corn oil or safflower oil. The effects of omega-3 PUFAs on arrhythmia deaths, ventricular fibrillation, ventricular premature beats, arrhythmia scores, infarct size, and length of time in sinus rhythm are reviewed below.

Effect on incidence of arrhythmia deaths. Seven studies in rats (Table 3-4) and one study in monkeys (Table 3-6) reported arrhythmia deaths. In the rat studies, investigators looked for deaths in ischemia-reperfusion-induced arrhythmias in 12 comparisons. In the monkey study, investigators looked for deaths after induced arrhythmias in 1 comparison (Table 3-6).

Meta-analyses of risk ratio of total deaths in ischemia-reperfusion-induced arrhythmias. As shown in Table 3-4, 12 comparisons in 7 studies were included in the meta-analyses. The 7 studies involved 150 rats that were fed omega-3 PUFAs and 152 rats fed omega-6 PUFAs. In all but one [17] of the studies, deaths during reperfusion after an ischemia procedure were monitored. Two studies [18,19] also looked for deaths that occurred during the ischemia procedure. They all found that deaths occurred only during the ischemia procedure; no deaths occurred during reperfusion in either the omega-3 PUFA or control groups The ischemia deaths in these two studies were combined into total deaths for ischemia-reperfusion-induced arrhythmia.

Of the 12 comparisons, 5 compared ALA oils to omega-6 PUFA oils (Figure 1). The combined risk ratio of deaths in ischemia-reperfusion-induced arrhythmias in these 5 comparisons was 1.2 (95% CI: 0.51-2.6). There was no statistically significant heterogeneity between studies.

The other 7 comparisons were combined to assess the effects of fish oils on deaths in ischemia-reperfusion-induced arrhythmias (Figure 2). The combined risk ratio of deaths in these 7 comparisons was 0.47 (95% CI: 0.23-0.93). There was no statistically significant heterogeneity between studies. However, the significantly reduced risk ratio of deaths was due to a single study [20] as shown by a sensitivity analysis (Table 3-5). When this study was removed, the combined risk ratio of deaths became 0.64 (95% CI: 0.19-2.1).

A separate meta-analysis combined comparisons involving ALA with comparisons involving eicosapentaenoic acid (EPA, 20:5 n-3) plus decosahexaenoic acid (DHA, 22:6 n-3). The overall risk ratio of deaths in this analysis was 0.68 (95% CI: 0.40-1.2).

Deaths from ventricular fibrillation in monkeys. One study examined total VF deaths— which combined deaths in the control condition, ischemia model, and isoproterenol model — among marmoset monkeys (Table 3-6). For the purpose of our evidence review, we evaluated only the results from a comparison between 16 monkeys fed fish oil and 13 monkeys fed sunflower seed oil. The fish oil and sunflower seed oil diets both had 12% weight-by-weight (w/w) of total fat or 29% kcal of fat. The fish-oil diet contained 2.8g/100g EPA plus DHA. The animals were fed for 30 months in both studies. No VF deaths occurred in the monkeys that were fed fish oil, while 3 deaths (23%) occurred in those fed sunflower seed oil.

Effects on incidence of ventricular tachycardia. Eight studies, representing 21 comparisons, reported the incidence of VT among rats fed omega-3 PUFA oils vs. those fed omega-6 PUFA oils (Table 3-7 and Table 3-8). In 10 of the comparisons, the incidence of VT in ischemia-induced arrhythmias was monitored (Table 3-7). In the other 11 comparisons, the incidence of VT during reperfusion-induced arrhythmias was monitored (Table 3-8). Only ischemia-induced VT was assessed in 2 [21,22] of the 8 studies. The remaining 6 studies assessed both ischemia-induced and reperfusion-induced VT.)

Meta-analyses of risk ratio of ventricular tachycardia in ischemia-induced arrhythmias. As shown in Table 3-7, 10 comparisons in 6 studies were included in the meta-analyses. Of the 248

rats used in the studies, 126 were in the omega-3 PUFA groups and 122 were in the omega-6 PUFA control groups.

Among the 10 comparisons, 4 examined the effects of ALA vs. omega-6 PUFA oils on the incidence of VT in ischemia-induced arrhythmias (Figure 3). The dose of ALA ranged from 0.4 to 5.2g/100g. The combined risk ratio of deaths was 0.82 (95% CI: 0.65-1.0). There was no statistically significant heterogeneity between studies.

The other 6 comparisons were combined to evaluate the effects of fish oils (EPA plus DHA) on the incidence of VT in ischemia-induced arrhythmias (Figure 4). The combined risk ratio of deaths in this analysis was 0.49 (95% CI: 0.29-0.83). The studies were heterogeneous. Sensitivity analysis did not show that any single study had a dominating effect.

A separate meta-analysis combined comparisons involving ALA with comparisons involving EPA plus DHA. In this meta-analysis, the overall risk ratio of VT in ischemia-induced arrhythmias was 0.70 (95% CI: 0.53-0.92).

Meta-analyses of risk ratio of ventricular tachycardia in reperfusion-induced arrhythmias. As shown in Table 3-8, 11 comparisons in 7 studies were included in these meta-analyses. Of the 257 rats used in the studies, 128 were in the omega-3 PUFA groups and 129 were in the omega-6 PUFA control groups.

Among the 11 comparisons, 5 examined the effects of ALA vs.omega-6 PUFA oils on the incidence of VT in reperfusion-induced arrhythmias (Figure 5). The combined risk ratio of deaths was 1.1 (95% CI: 0.73-1.6). The studies were heterogeneous.

The other 6 comparisons were combined to evaluate the effects of fish oils (EPA plus DHA) on the incidence of VT in reperfusion-induced arrhythmias (Figure 6). The combined risk ratio of deaths was 0.68 (95% CI: 0.50-0.91). There was no statistically significant heterogeneity between studies.

In the meta-analysis that combined comparisons involving ALA with comparisons involving EPA plus DHA, the overall risk ratio of VT in reperfusion-induced arrhythmias was 0.85 (95% CI: 0.65-1.1).

Effects on incidence of ventricular fibrillation. Nine studies in rats with 22 comparisons (Table 3-9 & Table 3-10), and 3 studies in monkeys with 9 comparisons (Table 3-11), reported the incidence of VF in induced arrhythmias. All the rat studies used ischemia-reperfusion models. In the monkey studies, arrhythmias were induced by electrical stimulation in normal or ischemic conditions and/or with the injection of isoproterenol.

In the rat studies, the incidence of VF in ischemia-induced arrhythmias was monitored in 8 comparisons (Table 3-9), while in the other 14 comparisons, the incidence of VF during reperfusion after an induced-ischemia procedure was monitored (Table 3-10). (Four [20-23] of the nine rat studies monitored only the incidence of reperfusion-induced VF. The remaining 5 studies monitored the incidence of both ischemia-induced and reperfusion-induced VF.)

Meta-analyses of risk ratio of ventricular fibrillation in ischemia-induced arrhythmias. As shown in Table 3-9, a total of 8 comparisons from 6 studies were included in the meta-analyses. Of the 176 rats used in the studies, 90 were in the omega-3 PUFA groups and 86 were in the omega-6 PUFA control groups.

Among the 8 comparisons, 3 examined the effects of ALA vs. omega-6 PUFA oils on the incidence of VF in ischemia-induced arrhythmias (Figure 7). The combined risk ratio of deaths was 0.95 (95% CI: 0.56-1.6). There was no statistically significant heterogeneity between studies.

The other 5 comparisons were combined to evaluate the effect of fish oils on the incidence of VF in ischemia-induced arrhythmias (Figure 8). The combined risk ratio of deaths was 0.21 (95% CI: 0.07-0.63). There was no statistically significant heterogeneity between studies.

In the meta-analysis that combined ALA comparisons and EPA plus DHA comparisons, the overall random-effect risk ratio of VF in ischemia-induced arrhythmias was 0.69 (95% CI: 0.41-1.24).

Meta-analyses of risk ratio of ventricular fibrillation in reperfusion-induced arrhythmias. As shown in Table 3-10, a total of 14 comparisons in eight studies were included in these meta-analyses. Of the 312 rats used in the studies, 155 were in the omega-3 PUFA groups and 157 were in the omega-6 PUFA control groups.

Among the 14 comparisons, 6 examined the effects of ALA vs. omega-6 PUFA oils on the incidence of VF in reperfusion-induced arrhythmias (Figure 9). The combined risk ratio of deaths was 0.84 (95% CI: 0.52-1.3). The studies were heterogeneous.

The other 8 comparisons were combined to evaluate the effects of fish oils on the incidence of VF in reperfusion-induced arrhythmias (Figure 10). The combined risk ratio of deaths was 0.44 (95% CI: 0.25-0.79). There was no statistically significant heterogeneity between studies.

In the meta-analysis combining ALA comparisons and EPA plus DHA comparisons, the overall random-effect risk ratio of VT in reperfusion-induced arrhythmias was 0.85 (95% CI: 0.65-1.1).

Ventricular fibrillation and ventricular fibrillation threshold in induced arrhythmia among monkeys. Table 3-11 shows results from 3 studies that compared monkeys fed fish oils to controls fed sunflower seed oil (omega-6 PUFA), and that examined the incidence of VF and ventricular fibrillation threshold (VFT) in induced arrhythmia. The dose of EPA plus DHA ranged from 1.8 to 2.8g/100g. The feeding duration ranged from 16 weeks to 30 months. Three different arrhythmia-induction protocols were used. In the first protocol, arrhythmias were induced by electrical stimulation in the control condition. In the second, arrhythmias were induced 5 minutes after an ischemia procedure, and in the third, arrhythmias were induced 30 minutes after restoration of coronary blood flow and during the infusion of isoproterenol. The three arrhythmia-induction protocols were not independent of each other, that is, the same monkeys underwent the series of experimental procedures in sequence. Thus, the cumulative effects of induced arrhythmias must be considered. (Note also be noted that the same group of investigators from one laboratory authored all three studies).

For each of the arrhythmia induction protocols, the investigators compared the proportion of monkeys from the fish oil group that experienced inducible VF to the proportion in the sunflower seed oil group that experienced VF. In the first protocol (electrical stimulation in control condition), the investigators found no difference between groups. In the second protocol (electrical stimulation five minutes after an ischemia procedure), 2 of the 3 studies found no difference in the proportion of monkeys that had inducible VF [24,25]. One study [26], however, reported that while no VF was inducible in the monkeys fed fish oil, VF was induced in 13% of the monkeys fed sunflower seed oil . In the third protocol (electrical stimulation during the infusion of isoproterenol), VF was induced in 30% to 50% of the monkeys fed fish oil compared to 77% to 100% of the monkeys fed sunflower seed oil.

Ventricular fibrillation thresholds (VFTs) were measured only among the VF inducible monkeys. In 2 studies[24,26], VFTs remained unchanged in both groups in all conditions. However, one study [25] found that VFTs were significantly increased among monkeys that were fed fish oil relative to those fed sunflower seed oil in all conditions (note that an increased threshold indicates a desirable outcome).

Effects on ventricular premature beats. As shown in Table 3-12, 7 studies with 16 comparisons evaluated the number of VPBs in ischemia-induced and/or reperfusion-induced arrhythmias. Rats were used in all 7 studies. No consistent results were found in the studies comparing rats fed ALA oils (soybean, linseed or canola oils) to rats fed omega-6 PUFA oils. However, studies comparing rats fed fish oils to rats fed omega-6 PUFA oils suggest that rats fed fish oils might have reduced numbers of VPBs in ischemia-induced and/or reperfusion-induced arrhythmias relative to rats fed omega-6 PUFA oils.

Effects on arrhythmia scores or severity of arrhythmias. As shown in Table 3-12, 8 studies with 18 comparisons evaluated the arrhythmia scores associated with the ischemia-induced and/or reperfusion-induced arrhythmias. Rats were used in all studies. More severe arrhythmias are associated with higher scores.

No consistent results were found in studies that compared rats fed ALA oils (soybean, linseed or canola oils) to rats fed omega-6 PUFA oils. However, when rats fed fish oils were compared to rats fed omega-6 PUFA oils, the studies found that most of the fish oil fed rats had less severe ischemia-induced and/or reperfusion-induced arrhythmias than the omega-6 PUFA fed rats.

Effects on infarct size. Infarct size, or size of the ischemic region, was evaluated in only 2 studies [16,27]. The results showed no significant difference in the infarct size between rats fed omega-3 PUFA oils and rats fed omega-6 PUFA oils (Table 3-12).

Effects on length of time in sinus rhythm. As shown in Table 3-12, 3 studies with 7 comparisons evaluated length of time in sinus rhythm (TSR) in ischemia-induced and/or reperfusion-induced arrhythmias. Rats were used in all studies. One study [27] that compared rats fed linseed oil (rich in ALA) to rats fed corn oil found no significant difference in TSR. In the same study, however, TSR was significantly increased in rats fed fish oil compared to rats fed corn oil. Two other studies compared rats fed fish oils to rats fed omega-6 PUFA oils. These studies found no significant difference in TSR in ischemia-induced and/or reperfusion-induced arrhythmias [19,28].

Studies Comparing Pre-fed Omega-3 Long-Chain PUFAS to a-Linolenic Acid

Two studies directly compared omega-3 long-chain PUFAs (EPA and DHA) to ALA (Table 3-13). Both studies found a non-significant reduction in the incidence of VT and VF in ischemia-induced or reperfusion-induced arrhythmias in rats fed fish oils compared to those fed soybean or linseed oils (rich in ALA) [23,27]. Abeywardena et al. found that no deaths occurred in rats fed fish oil, while 11% of rats fed soybean oil died from ischemia-reperfusion-induced arrhythmias [23]. The results also showed that rats fed fish oil had fewer numbers of VPBs and less severe arrhythmias as indicated by arrhythmia score than did rats fed soybean oil. However, none

of these results were statistically significant. In addition to the incidence of VT and VF, Isensee et al. [27] also examined the infarct size and the length of time in normal sinus rhythm. The results showed no difference in infarct size between rats fed a fish oil diet and those fed a linseed oil diet after 20 minutes of ischemia. The length of time in normal sinus rhythm was almost 50% longer in rats fed fish oil compared to rats fed linseed oil.

Indirect comparisons between omega-3 long chain PUFAs (EPA and DHA) and ALA based on meta-analysis are described in the Discussion (Chapter 4).

Studies Comparing Pre-fed Omega-3 PUFAs to MUFAs

One study [29] compared the anti-arrhythmic effects of PUFAs to those of MUFAs (see Evidence Table 1). In this study, rats that were fed EPA, DHA, or a mixture of EPA and DHA were compared to rats that were fed olive oil. All animals used in the study were male, spontaneously hypertensive strains (n=10 per group). All synthesized diets contained 5% total fat, which represented 12% of available energy as fat. The rats underwent a common surgical procedure to induce myocardial ischemia after eating synthetic diets for 5 weeks. Results showed that DHA and EPA plus DHA significantly reduced the incidence and severity of ventricular arrhythmias as indicated by the arrhythmia score; however, EPA alone had no effect. Ventricular fibrillation occurred in 80% of the rats who were fed olive oil, 70% of those fed EPA, 20% fed DHA, and 10% fed the mix of EPA plus DHA. Compared to the controls, the incidence of VF was significantly lower in the DHA-fed rats ($P<.01$) and in the rats fed the mix of EPA plus DHA ($P<.01$). However, VF was not significantly lower in rats fed the EPA diet.

Studies Comparing Pre-fed Omega-3 PUFAS to Saturated Fatty Acids

As shown in Table 3-1, we analyzed 5 studies that compared omega-3 PUFAs to saturated fatty acids. In each study, experimental and control oils were added to the animals' basic diets in equal amounts (see Evidence Table 1). Therefore, all comparisons reflect iso-caloric intake from fat. Fish oils and sardine or mackerel oils were used as the source of omega-3 PUFAs in the experimental groups, while controls were fed coconut, lard, sheep peri-renal fat, or butter. The dosages of EPA plus DHA were 0.6g/100g [30], 2.9g/100g [31], 5.5g/100g [32] and 5%kcal [33,34].

Effects on incidence of arrhythmia deaths. As shown in Table 3-14, deaths in ischemia-reperfusion-induced arrhythmias were monitored in 2 studies. In 1 study [33], rabbits fed fish oil corresponding to a dose of EPA plus DHA of 5.2g/100g were compared to controls fed coconut oil. The animals were fed for 12 weeks before arrhythmias were induced. In one arm of the study, animals were subjected to 10 minutes of ischemia followed by one hour of reperfusion. Three deaths (25%) were observed among the 12 rabbits fed fish oil, compared to three deaths (36%) among the 14 rabbits fed coconut oil. Two of the deaths in both groups occurred during reperfusion. In another arm of the study, rabbits were subjected to 1 hour of ischemia followed by 4 hours of reperfusion. Six deaths (43%) were observed among the 14 rabbits fed fish oil, compared to 8 deaths (53%) in the 15 rabbits fed coconut oil. About 50% of the deaths occurred during ischemia and 50% occurred during reperfusion in both groups in this arm.

In another study [30], 13 piglets were fed either 9%w/w lard fat (n=6) or 4.5%w/w mackerel oil plus 4.5%w/w lard fat (n=7) for 16 weeks. The corresponding dose of EPA plus DHA in the group

fed mackerel oil plus lard fat was 0.6g/100g. Defibrillation was unsuccessful in one piglet from the mackerel plus lard oil group. This piglet died of ventricular asystole during the fifth reperfusion.

Effects on incidence of ventricular tachycardia. As shown in Table 3-15, 2 studies examined the incidence of VT in ischemia-reperfusion-induced arrhythmias. One of these studies was in rats [35], and 1 was in piglets [30]. In the rat study, 7 (35%) of the 20 rats fed fish oil developed VT, compared to 14 (70%) of the 20 rats fed sheep peri-renal fat ($P<.05$).

In the piglet study, the incidence of VT was 29% (n=7) and 17% (n=6) in piglets fed mackerel oil and lard fat, respectively. All VT events occurred during the ischemia procedure.

Effects on incidence of ventricular fibrillation. As shown in Table 3-16, 3 studies examined the incidence of VF in ischemia-reperfusion-induced arrhythmias. Two of these studies were in rats [34,35] and 1 was in piglets [30]. Both rat studies found a significantly reduced incidence of VF in ischemia-reperfusion-induced arrhythmias among rats fed fish oil compared to rats fed saturated fats.

In the piglet study, the incidence of VF was 43% (n=7) in piglets fed mackerel oil, while no piglet fed lard fat developed VF in ischemia-reperfusion-induced arrhythmias. In the same study, programmed electrical stimulation was performed to induce VF in another 20 piglets, 10 in the mackerel-oil group and 10 in the control group. The incidence of VF was not reported, but VFTs were measured in control condition and during 15 minutes of ischemia. The threshold current for VF induction was reduced in all dietary groups during ischemia but remained significantly higher in the mackerel-oil-fed group than in the saturated-fat-fed group.

Effects on ventricular premature beats. As shown in Table 3-17, 3 studies examined the number of VPBs in schemia-reperfusion-induced arrhythmias. One of these studies was in rabbits [33], 1 was in piglets [30], and 1 was in rats [35]. In the rat study, the number of VPBs during ischemia was significantly reduced among rats fed fish oil compared to rats fed sheep-perirenal fat. In the piglet study, the incidence of VPBs during ischemia did not differ between the groups. However, during reperfusion the piglets fed mackerel oil had significantly fewer VPBs compared to those fed lard fat. In the rabbit study, there were no significant differences in the incidence of VPBs between rabbits fed fish oil and those fed coconut oil during ischemia or reperfusion. However, rabbits that died from arrhythmias were excluded from the analyses, and more rabbits died in the control group than in the experimental group. Thus, the true effects were underestimated.

Effects on arrhythmia scores or severity of arrhythmias. None of the studies that compared the arrhythmic effects of omega-3 PUFAs and saturated fatty acids reported arrhythmia scores as an outcome.

Effects on infarct size. None of the studies that compared the arrhythmic effects of omega-3 PUFAs and saturated fatty acids reported infarct size as an outcome.

Effects on length of time in sinus rhythm. None of the studies that compared the arrhythmic effects of omega-3 PUFAs and saturated fatty acids reported length of time in sinus rhythm as an outcome.

Studies Comparing Pre-fed Omega-3 PUFAS to No Treatment Controls

A total of 4 studies were included in this analysis (Table 3-1). As shown in Evidence Table 1, omega-3 PUFA oils were added to the diet of animals in the experimental groups, while controls were maintained on basic diets. As a result, energy intake from fat was higher in the experimental groups than in the control groups. Dogs were used in all studies. MaxEPA, menhaden oil, or EPA esters were used as the source of omega-3 PUFAs in the experimental groups, while controls were fed standard dog chows (Oriental Yeast Co. or Friskies® Dinner). The dose of EPA plus DHA was 3.3%kcal [36] and 1.0g/100g [37] in the two fish-oil studies. The dose of EPA was 1.0g/100g in both of the EPA-ester studies [38,39].

Effects on incidence of arrhythmia deaths. Two studies [36,39] that evaluated the incidence of arrhythmia deaths (Table 3-18) compared dogs fed EPA and/or DHA to no treatment controls. In one study, no significant difference was found in the incidence of sudden death after induced-coronary thrombosis. [36] The other study found no deaths due to VF in the 10 dogs fed 1.0g/100g EPA ester, although five VF deaths (33%) occurred in the 15 untreated control dogs ($P<.05$). [39].

Effects on incidence of ventricular tachycardia and/or ventricular fibrillation. The incidence of VT and/or VF in induced arrhythmias was evaluated in a study of 30 dogs. [38]. Fifteen dogs were fed standard dog chow plus 1.0g/100g EPA ester for 8 weeks. Fifteen untreated control dogs were fed standard dog chow for 8 weeks. An ischemia-induced arrhythmia model was used in 10 experimental dogs and 10 controls. A digitalis-induced arrhythmia model was used in 5 dogs from each group. A fatal dose of digoxin (0.025 mg/kg/min) was administrated intravenously over a 60-second period immediately after coronary artery ligation.

There was no difference in the incidence of VF in ischemia between the groups. Two dogs in each group (20% vs. 20%) developed VF within 3 hours after coronary ligation. All 10 dogs that underwent digitalis-induced arrhythmias developed VT or VF. However, the VT or VF did not occur until at least 25 minutes after the administration of digoxin in the dogs fed EPA ester, while the events occurred about 10 to 15 minutes within administration of digoxin in the untreated control dogs.

Effects on ventricular premature beats. As shown in Table 3-19, 2 studies [36,38] examined the number of VPBs in induced arrhythmias. Both studies found that dogs fed EPA and/or DHA had fewer VPBs compared with untreated controls.

Effects on arrhythmia scores or severity of arrhythmias. One study [38] evaluated the arrhythmia score in ischemia-induced arrhythmias (Table 3-19), and found that the arrhythmia score obtained within 3 hours after coronary ligation was significantly reduced by EPA-supplementation. Dogs fed EPA esters for 8 weeks had significantly less severe ischemia-induced arrhythmias than the no treatment controls ($P<.01$).

Effects on infarct size. Infarct size, or size of the ischemic region, was evaluated in 3 studies (Table 3-19) [36,37,39]. All 3 studies showed that dogs fed EPA and/or DHA had a decrease in the infarct size in either electrical-stimulation-induced or ischemia-reperfusion-induced arrhythmias

compared to untreated controls. However, areas at risk of arrhythmias were not significantly different between groups.

Effects on length of time in sinus rhythm. None of the studies that compared the arrhythmic effects of omega-3 PUFAs vs. untreated controls reported length of time in sinus rhythm as an outcome.

Anti-arrhythmic Effects of Free Omega-3 Fatty Acids

Three studies examined the effects of intravenously infused omega-3 fatty acids on ischemia-induced or spontaneous arrhythmias (Table 3-20). The fatty acids were infused in their free form bound to albumin. Dogs were used in all studies. Controls received infusions of saline or buffer, or of soybean lipid emulsion with 7-8% ALA. Cardiac function was monitored by ventricular electrocardiography. Because cardiac response was similar among the control groups, data for control dogs were combined if 2 groups of controls were used.

Effects on incidence of ventricular tachycardia and ventricular premature beats. One study [40] evaluated the incidence of VT in spontaneous arrhythmias in 8 dogs (Table 3-20). The dogs were first injected with control buffer. Data obtained after this injection served as controls. After all hemodynamic parameters had completely recovered, the same protocol was used to infuse the dogs with various doses of ALA: 1 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, or 60 mg/kg. No VT or VPB events were observed when infusing the control buffer or when infusing up to 10 mg/kg of ALA. However, at doses of 20 mg/kg, 30 mg/kg, and 60 mg/kg of ALA, the incidence of VT was 13%, 38%, and 63%, respectively. The effects of ALA on the number of VPBs was similar. However, possible cumulative effects are of concern in this study since the experiments were not independent of one another.

Effects on incidence of ventricular fibrillation. As shown in Table 3-20, 2 studies [41,42] evaluated the incidence VF in exercise-plus-ischemia-induced arrhythmias. The results showed fish-oil emulsion, or albumin-bound EPA-, DHA-, or ALA-concentrates significantly reduced the incidence of VF.

Whole-Animal Isolated Organ and Cell Studies

In this section, we present the results of 21 studies that examined the effects of omega-3 fatty acids in isolated organs and cells from whole animals. In these studies, omega-3 fatty acids were fed to whole, intact animals as part of their diet, and organs or cell tissues were subsequently excised from the animal for study. The effects of omega-3 fatty acids on the following parameters are discussed: contractile parameters, basoelectromechanical parameters, ion pumps and ion movements, ion currents, and ion channels. Tables 3-20 through 3-24 and Evidence Table 2 contain the results for this section.

Contractile Parameters

Eight studies evaluated the effect of diets enriched with omega-3 fatty acids on contractile parameters such as heart rate, contraction rate, contraction amplitude, diastolic and systolic cell length, percent cell length, post-rest potentiation, and cardiac work. All studies used rat models. The developmental stage of the rats, however, varied considerably (2 weanling; 2 young adults; 3 adults; 1 aged). See Table 3-21.

Heart rate. Under ambient conditions and in the absence of any agent, 3 studies showed that fish oil or EPA/DHA supplementation did not change heart rate [43-45]. One study showed that in the presence of the arrhythmogenic agent lipopolysaccharide (LPS), fish oil significantly decreased heart rate compared to a safflower oil diet [43]. One study examined the effect of cod liver oil supplementation on heart rate under various conditions. In the absence of nor-adrenalin under high oxygenation, there was a significant decrease in heart rate, but there was no change in the presence of nor-adrenalin. In the presence of nor-adrenalin under hypoxic conditions, there was a significant decrease in heart rate. Upon re-oxygenation, there was no change. [46]. See Table a 3-21.

Contractility. Two studies by the same author compared the effects of fish oil supplementation, safflower oil, and lard on contraction rate induced by isoproteronol (ISO) and free radical generating system (FRGS) [47,48]. Both studies found a significant decrease in contraction rate among the fish oil group. Another study compared the effects of fish oil and safflower oil on force of contraction, maximum rate of rise of contraction, and maximum rate of relaxation. This study found no change in any of the parameters in the presence of saline, but found a significant increase in all parameters in the presence of lipopolysaccharide [43]. One study measured force-velocity relationship characteristics following consumption of an N-3 fatty acid diet vs. an N-6 diet and showed no change [49]. See Table 3-21.

Ionotropic parameters. One study examined the effect of fish oil versus lard treatment on diastolic and systolic cell length, percent cell length, and post-rest potentiation, and showed no change in these parameters [48]. Another study measured amplitude of contraction under various experimental conditions. In the absence of nor-adrenalin under high oxygenation, there was a significant decrease in amplitude of contraction, but there was no change in the presence of nor-adrenalin. In the presence of nor-adrenalin under hypoxic conditions, there was a significant decrease in amplitude. Upon re-oxygenation, there was no change [46]. See Table 3-21.

Cardiac work. One study compared the effects of linseed oil treatment and sunflower oil treatment on cardiac work and reported no difference between the two groups [50]. See Table 3-21.

Basoelectromechanical Parameters

Three studies examined the effect of omega-3 fatty acids on basoelectromechanical parameters in whole animal isolated organs and cells. One study used a rat model and showed that supplementing a high fat diet with fish oil significantly reduced the ventricular effective refractory period [45]. Another rat model study reported no change in developed or resting tension in the isolated perfused heart after cod liver oil supplementation [51]. The third study used a rabbit model and showed no effect of dietary fish oil compared to safflower oil on the ventricular effective

refractory period, absolute refractory period, relative refractory period, or epicardial or endocardial monophasic action potential [52].
See Table 3-22.

Ion Pumps and Ion Movement

Fourteen studies examined the impact of omega-3 fatty acid enriched diets on ion pumps and ion movement (IPIM) in whole animal isolated organs and cells. Three studies used mouse models, 8 used rat models, 1 used rabbit models, 1 used pig models, and 1 used a canine model. See Table 3-23.

Pump activity. Eight studies examined either calcium-magnesium ATPase or sodium-potassium ATPase activity in isolated organs and cells from whole animals. Among the 3 studies that used mouse models, one study compared diets enriched with EPA ester or DHA to a diet containing safflower oil and found no change in sarcoplasmic reticulum calcium-magnesium ATPase activity with either the EPA ester or DHA ester diet [53]Croset, 1989b]. A second mouse study compared a diet rich in fish oil to one rich in corn oil and found a significant decrease in sarcoplasmic reticulum calcium-magnesium ATPase activity with the fish oil diet[54]. The third mouse study showed that, compared to a standard chow diet, supplementation with graded doses of DHA ester did not affect calcium-magnesium ATPase activity in the SR, but at low doses it significantly increased calcium-magnesium ATPase in the cardiac myocyte. At a higher dose, however, there was no change [55]. Two studies used a rat model. One compared a fish oil diet to a corn oil diet and used a graded dose of ATP and ionomycin, and measured sarcoplasmic reticulum calcium-magnesium ATPase, calcium ATPase, and magnesium ATPase. This study found significant decreases in these parameters [56]. A study using a canine model, reported significant increases in cardiac calcium-magnesium ATPase with EPA ester supplementation [38]. Three studies (2 rat and 1 canine model) all reported no change in sodium-potassium ATPase activity with an omega-3 fatty acid diet, regardless of dosage or agent used [38,57,58]. One study using a pig model reported significant increases in calcium pumping ATPase activity after consumption of a fish oil vs. a lard-enriched diet both under ambient and ischemia-reperfusion conditions [59]. See Table 3-23.

Cytosolic calcium influx. Two studies using rat models measured cytosolic calcium influx. One reported a significant increase in cytosolic calcium influx under ischemic conditions with a cod liver oil diet [51]. Another study compared fish oil to canola oil and reported no change under ambient conditions in cytosolic calcium (Ca^{2+}) influx [60]. See Table 3-23.

Cytosolic calcium efflux. Only one study compared cod liver oil supplementation to a standard chow diet using a rat model under ischemic reperfusion conditions. That study reported no change in cytosolic calcium efflux under ischemia reperfusion conditions [51]. See Table 3-23.

Cytosolic calcium content. Three studies using rat models examined the effect of fish oil supplementation on cytosolic calcium content. In comparison to an omega-6 or saturated fatty acid diet, fish oil supplementation demonstrated no effect under ambient conditions in any of the studies [32,33,47]. Two of these studies examined the effect of fish oil under ischemic/reperfusion conditions. One study found no change [33], while the other reported a significant decrease in

cytosolic calcium content which was more pronounced in aged (vs. younger) rats [61]. See Table 3-23.

Sarcoplasmic reticulum calcium content. Three studies (one mouse and two rat models) examined the effect of fish oil supplementation on sarcoplasmic reticulum calcium content. Two of the studies showed a significant decrease in sarcoplasmic reticulum calcium content. One of the 2 studies compared ALA or EPA or DHA ester to a safflower oil control, while the other compared fish oil supplementation to a corn oil diet [53,56]. The third study compared fish oil supplementation to a diet enriched with saturated fats and reported no difference in caffeine or 2,4-Di-tert-butylhydroquinone (DBHQ)-induced alterations in sarcoplasmic reticulum calcium content with fish oil supplementation compared to one enriched with saturated fats [47]. See Table 3-23.

Sarcoplasmic reticulum calcium uptake. Two studies (one mouse and one rat model) compared the effects of fish oil supplementation vs. corn oil on sarcoplasmic reticulum calcium uptake. Both studies showed a significant decrease in sarcoplasmic reticulum calcium uptake among rats receiving fish oil supplementation [54,56]. Another study used a rat model to compare fish oil supplementation to a saturated fat diet. This study reported a significant increase in sarcoplasmic reticulum calcium exchanger or sarcoplasmic reticulum efflux induced by DBHQ or isoproteronol [47] among the rats receiving fish oil. One study comparing the effect of fish oil supplementation to a standard chow diet demonstrated no change in sarcoplasmic reticulum calcium transport activitiy using a rat model[62]. See Table 3-23.

Ion Currents

Two studies examined the effect of omega-3 fatty acid diet supplementation on ion currents in isolated organs and cells from whole animals. Both studies used rat ventricular myocytes, and both studies compared a fish oil diet to a high fat diet [48,63]. See Table 3-24.

Sodium currents. One study measured sodium currents (I_{NA}) and reported no change in either activation or inactivation parameters [48]. See Table 3-24.

Transient outward currents.
One study measured transient potassium outward currents (I_{to}) and reported no change in either activation or inactivation parameters [48]. See Table 3-24.

Voltage dependent L-type calcium current. One study measured voltage dependent L-type calcium current (ICa.L) and observed no change in activation parameters, inactivation parameters, or amplitude of voltage dependent L-type calcium current.[63]. See Table 3-24.

Ion Channels

Two studies evaluated the effect of omega-3 fatty acid diet supplementation on ion channels in whole animal isolated organs and cells. Rat models were used in both studies. One of the studies measured in ventricular crude sarcolemma preparations the binding site affinity and affinity (K_d)

of [^{3}H] nitrendipine for the calcium channels. There was no change reported in either B_{max} or K_d attributable to cod liver oil in adult rats. A similar result was observed in aged rats. When aged rats were compared to adult rats, there was a significanty lower K_d in the aged rats [64]. A second study comparing fish oil to a high fat diet assessed the binding characteristics of the phenylalkylamine (PAA) receptor with verapamil and those of the benzonthiazepine (BT) receptor with diltiazem and reported no change on the parameters of the calcium current-voltage (I_{Ca}–V) curves [63]. See Table 3-25.

Isolated Organ and Cell Culture Studies

In this section, we present the results of 39 studies that examined the effects of omega-3 fatty acids on isolated organs and cells extracted from whole animals. Twenty-nine of these studies used rat models, 1 used a mouse model, 2 used guinea pig models, 2 used dog models, 1 used a ferret model, 1 used a pig model, and 1 used a cat model. Two studies used both rat and guinea pig models. Tissues and organelles extracted for analysis included the whole heart, ventricular or atrial cardiomyocytes, sarcolemmal or microsomal vesicles, and myocardial or ventricular mitochondria. The omega-3 fatty acids tested in these studies included ALA, EPA, DHA, or their combination. The omega-3 fatty acids were applied either directly to the cell culture medium (free) or incubated with the cells to allow incorporation into membrane phospholipids (bound). Each row of the summary tables represents a comparison using the following factors: study, diet, free or bound fatty acid, dosage, experimental condition (ambient, hypoxia, reoxygenation) or agent used. Tables 3-26 through 3-31 and Evidence Table 3 contain the results for this section.

Contractile and Arrhythmogenic Parameters

This section summarizes 22 studies that examined the effect of omega-3 fatty acids on arrhythmogenic and contractile parameters in isolated organs or cells. In 11 of these 22 studies, the omega-3 fatty acids were free, and in 9 studies the cells were bound with the fatty acids. Two studies employed both approaches. Nineteen studies used rat models, 2 used guinea pig models, and 1 used both a rat and guinea pig model. See Table 3-26.

Arrhythmias. Seven studies examined the effect of omega-3 fatty acids on arrhythmias. Arrhythmias were defined as spontaneous or asynchronous contractions induced by various agents. Four of the studies using rats were from the same group of collaborators and demonstrated that free EPA or DHA significantly prevented or terminated the proportion of arrhythmias induced by ouabain, calcium, lysophosphatidylcholine (LPC), palmitoylcarnitine (PTC), or eicosanoids [65-67,67,68]. Another study by the same collaborative group examined the effect of free and bound EPA or DHA in a rat model, and demonstrated that free but not bound omega-3 fatty acids were effective in terminating induction of arrhythmias [69]. Another study using a rat model showed that bound DHA significantly decreased the proportion of arrhythmias induced by nor-adrenaline and timolol (TIM) [70]. A study using a guinea pig model showed that free EPA (sodium salt) at a low dosage did not have an effect on antigen-induced arrhythmia but produced a significant decrease in the proportion of induced arrhythmias at a high dosage [71]. See Table 3-26.

Contractility. Eighteen studies examined the effect of omega-3 fatty acids on contractility parameters such as contraction rate (spontaneous or induced), contraction frequency, electrical automaticity/excitability (EA) , diastolic length (DL), twitch amplitude (TA), velocity of shortening /diastolic length (VS/DL), and twitch size (TS) both in the presence and absence of several arrhythmogenic agents. Fourteen studies used rat models, 2 studies used guinea pig models, and 2 studies used both rat and guinea pig models. See Table 3-26.

In 13 of these studies the effects of the omega-3 fatty acids were studied compared to a control group; in 1 study the comparison group was a saturated fatty acid, in 2 studies the comparison group was either control or an omega-6 fatty acid, and in 2 studies (by the same author) the comparison group was another omega-3 fatty acid. The results are discussed based on the comparison group and agent used. See Table 3-26.

In the contractility studies that tested the effect of free ALA, EPA, DHA, or a combination compared to control in the absence of any agent, 3 showed no effect [65,72,73], while 3 showed a decrease [66,74,75]. The following arrhythmogenic agents were examined: ouabain, nitrendipine, Bay8644 (BAY), isoproteronol, LPC, dibutyryl cyclic adenosine monophosphate (dBcAMP), eicosanoids, high extracellular calcium, and cholera toxin. All studies reviewed, regardless of species used, demonstrated a decrease in contractility or a protective effect of the omega-3 fatty acids in blocking the negative response induced by the agents [65-68,72,73,76,77,78]. One study also showed that DHA blocked the inhibitory effect of nitrendipine on myocyte contraction but not the inhibitory effect of verapamil and diltiazem on myocyte contraction [65]. See Table 3-26.

One study examined the effect of free DHA versus the saturated fatty acids docosanoic acid and stearic acid in the presence of LPC or isoproteronol in a rat model and observed a significant decrease in both spontaneous and asynchronous contractility [79]. Two studies examined the effect of a combination of either free ALA+EPA [73] or bound EPA+DHA [80] compared to an omega-6 fatty acid and found no difference in contractility in the absence of an arrhythmogenic agent. In the presence of arrhythmogenic agents (isoproteronol and phenylephrine [PHE]), one study showed no effect of free ALA+EPA [73], while the other study observed a significant increase with bound EPA+DHA [80]. See Table 3-26.

In 2 studies [11,81] of bound EPA compared to bound DHA (omega-3 vs omega-3), there was no effect on frequency of spontaneous contractions in the absence of an agent or with PHE. However, in the presence of an agent such as ISO or dBcAMP, bound EPA was significantly more effective than bound DHA in reducing the frequency of spontaneous contractions. See Table 3-26.

Three studies also examined the effect of methylated (m.e.) or ethylated (e.e.) free EPA or DHA on contractility. Two of these studies were performed using rat models and showed that free EPA e.e. in the absence of an agent, or free DHA m.e. in the presence of ISO, had no effect on contractility [66,76]. The third study, which used a guinea pig model, showed that free DHA methyl ester (m.e.) significantly increased calcium-induced calcium release (CICR) contractions but not voltage-sensitive release mechanism (VSRM) contractions [82]. See Table 3-26.

One study examined the effect of free DHA on DL, TA, and VS/DL in a rat model and showed no effect in the absence of an agent or ISO, but produced a blockade with the addition of nitrendipine or BAY [61]. See Table 3-26.

Two studies examined the effect of omega-3 fatty acids on twitch size, and both used rat and guinea pig models [77,83]. A decrease in twitch size with free EPA and/or free DHA was observed in both guinea pig studies. In the studies using rats, 1 study observed an increase in twitch size with EPA or DHA at concentrations between 1-7.5µm, and decreases in twitch size with concentrations

>10µm [83]. In the other rat study, 5 µm of EPA significantly decreased twitch size [77]. See Table 3-26.

Inotropic parameters. Three studies examined the effect of omega-3 fatty acids on inotropic parameters. One study using a rat model reported that neither free EPA nor DHA had an effect on amplitude of contraction [66]. Free EPA significantly increased resting cell length in another study using a rat model [74]. A third study using bound EPA with a rat model showed no change in amplitude but a significant increase in amplitude with ouabain [72]. See Table 3-26.

Other contractility parameters. Seven studies using rat models (3 by the same investigator[11,81,84] examined the effect of bound omega-3 fatty acids on the following contraction parameters: contraction coupling delay (tC_{20}), contraction duration at 20% relaxation (CD_{20}), contraction duration at 80% relaxation (CD_{80}), relaxation time (-Cmax), and cell shortening velocity (+Cmax). See Table 3-26.

Two of these studies examined the effect of bound omega-3 compared to bound omega-6 fatty acids under 3 conditions — ambient, hypoxia, and reoxygenation — and showed no effect on the contractility parameters that were investigated [85]. Four studies (2 from the same laboratory) compared bound EPA to DHA and found no difference in their effects on CD_{20}, CD_{80}, –Cmax, and +Cmax, regardless of the agents used to induce arrhythmia [80,81,84,86]. One study compared bound ALA+EPA to omega-6 fatty acids and reported no difference in CD_{80} and –Cmax but found a significant increase in +Cmax [73] with ALA+EPA. The presence of ISO did not alter the effect of ALA+EPA on these parameters. See Table 3-26.

Basoelectromechanical Parameters

This section summarizes 9 studies (4 from the same group of collaborators) [11,84-86] that examined the effects of omega-3 fatty acids on basoelectromechanical parameters in isolated organs and cells. Seven of these studies used rat models. One study used both a rat and guinea pig model, and 1 used a cat model. Free omega-3 fatty acids were used in 3 rat studies, in the study using both rat and guinea pig models, and in the study using the cat model. Bound omega-3 fatty acids were used in 4 of the studies using rat models. See Table 3-27.

The single study that used a feline model examined the effect of free ALA on four basal electric parameters not measured by any of the other researchers — intra-atrial conduction time, atrioventricular conductance time, atrial functional refractory period, and functional refractory period of the atrioventricular conducting system [87]. No changes were observed in any of these parameters. See Table 3-27.

Action potential. Six studies using rat models examined the effect of omega-3 fatty acids on the action potential. One reported an increase [88] with free EPA compared to a control, while another study, also using free EPA, reported a significant decrease in both the action potential and the frequency of the action potential [89]. See Table 3-27.

In the presence of 3 different agents (sodium and timolol [TIM], isoproteronol, and ouabain), bound DHA was shown to significantly decrease the action potential compared to control. No change was observed in the absence of an agent [70]. Two studies compared bound synthesized medium for omega-3 group (SM3) to bound synthesized medium for omega-6 group (SM6) and

reported no change in the action potential under ambient, hypoxic, and reoxygenated conditions [84,85]. See Table [3-27].

A study that compared bound EPA to bound DHA also found no difference in effect [86]. See Table 3-27.

Action potential amplitude. Seven studies examined the effect of omega-3 fatty acids on the amplitude of the action potential. All studies used rat models. Two studies showed that 5-10µM of free EPA and/or DHA did not affect the action potential amplitude (APA) compared to control [88,89], but concentrations >10-50 µM showed a significant decrease [88]. One study compared the effect of bound DHA relative to control and reported a significant increase in action potential amplitude using EPA [70]. See Table 3-27.

Two studies examined the effects of omega-3 fatty acid combinations (SM3) versus omega-6 fatty acids (SM6), under varying conditions. Both showed no change in APA under ambient conditions and a significant decrease in APA under hypoxic conditions. Under the reoxygenation condition, however, the results differed; one study reported no change [85] and the other reported a significant increase in action potential amplitude [11]. See Table 3-27.

Two studies compared the effect of bound EPA to bound DHA and found that EPA significantly increased APA compared to DHA [11,86]. See Table 3-27.

Action potential duration at 40% depolarization. Four studies using rat models examined the effect of omega-3 fatty acids on the action potential duration at 40% polarization. One study reported an increase in this parameter in the presence of both free EPA and free DHA compared to control [88]. See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions, 1 reported no change under all 3 conditions [84], while the other reported a significant decrease in action potential duration at 40% polarization under hypoxic conditions with SM3, but no change under ambient or reoxygenation conditions for [85]. See Table 3-27.

One study comparing bound EPA to bound DHA did not find a differential effect on this basal electromechanical parameter [86]. See Table 3-27.

Action potential duration at 80% depolarization. Five studies using rat models and 1 study using both a rat and guinea pig model examined the effect of omega-3 fatty acids on the action potential duration at 80% polarization (APD_{80}). One study using free EPA (10µM) compared to control, reported a significant decrease in the action potential duration [89]. Similarly, another study reported a dose dependent decrease in action potential duration at 80% polarization with EPA concentrations >10µM but an increase with EPA concentrations between 1-7.5µM [83]. The same authors also used a guinea pig model and reported that EPA was effective in decreasing action potential duration at 80% polarization at concentrations between 1-20µM. See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions, 1 reported no change under all 3 conditions [84], while the other reported a significant decrease in action potential duration at 80% polarization under hypoxic conditions, but no change under ambient or reoxygenation conditions [85]. See Table 3-27. Two studies compared bound EPA to bound DHA and observed no effect on the action potential [11,86]. See Table 3-27.

Maximum rate of depolarization. Six studies using rat models examined the effect of omega-3 fatty acids on the maximum rate of depolarization (V_{MAX}) of the action potential. One

study showed a decrease in V_{max} with either free EPA or free DHA compared to control [88]. See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions. One reported no change under any of the 3 conditions [84], while the other reported a significant increase in V_{max} under ambient conditions, but observed no change under either hypoxic or reoxygenated conditions [85]. See Table 3-27.

Two studies compared bound EPA to bound DHA and found no difference in V_{MAX} [11,86]. See Table 3-27.

Maximum diastolic potential. Four studies using rat models examined the effect of omega-3 fatty acids on the maximum diastolic potential (MDP). See Table 3-27.

Two studies compared bound SM3 to bound SM6 under varying experimental conditions, and observed that SM6 did not affect MDP under ambient and hypoxic conditions [84,85]. However, under reoxygenation conditions, one study showed an improvement [85] while the other showed no change [84]. Two studies compared bound EPA to bound DHA and both reported no change in MDP [11,86]. See Table 3-27.

Overshoot potential. Four studies (all by the same collaborative group) using rat models examined the effect of omega-3 fatty acids on the overshoot potential (OS). A study comparing bound SM3 to bound SM6 reported no effect on OS [85]. Another study also compared bound SM3 to SM6 but under varying experimental conditions, and found that SM3 did not affect OS differently from SM6 under ambient conditions, but significantly decreased OS under hypoxic conditions and significantly increased OS during reoxygenation [84]. See Table 3-27. Two studies comparing bound EPA to bound DHA reported that EPA significantly increased OS compared to DHA [11,86]. See Table 3-27.

Other basoelectromechanical parameters. In a cat model infusion of ALA in the presence of indomethacin there was no change in the following basoelectrical parameters such as AC, AVC, ARP, and AVRP.

Ion Pumps and Ion Movements

This section summarizes 13 studies that examined the effects of omega-3 fatty acids on ion pumps and ion movements in isolated organs and cells. In 10 of these studies, the omega-3 fatty acids were applied directly in free form, and in 2 studies the cells were incubated with the fatty acids to allow incorporation into membrane phospholipids (bound). In 1 study both approaches were used. Nine studies used rat models, 2 used canine models, 1 used a pig mode1 used both a rat and guinea pig model. See Table 3-28.

Pump activity. One study, which used a rat model, examined the impact of bound EPA on pump activity (sodium-potassium ATPase). This study reported no effect in the presence of ouabain or bumetanide (BUME), or with a combination of these two agents [72]. See Table 3-28.

Cytosolic calcium influx. Three studies examined the effect of omega-3 fatty acids on cytosolic calcium influx. The first used a rat model and reported that free EPA decreased cytosolic calcium influx [90]. In the second study, free DHA blocked the effect of nitrendipine and BAY on

cytosolic calcium influx [61]. Another study using a rat model examined the effect of bound EPA or bound DHA in the presence of several agents and found that DHA blocked the ouabain-induced increase in cytosolic calcium influx. Both EPA and DHA blocked the nitrendipine (NIT)-induced decrease, ouabain+nitrendipine-induced decrease, BAY+nitrendipine-induced decrease, and the BAY-induced increase in cytosolic calcium influx [65]. See Table 3-28.

Cytosolic calcium efflux. One study using a rat model examined the effect of free EPA on cytosolic calcium efflux in the presence of either calcium or caffeine and demonstrated no effect [90]. See Table 3-28.

Cytosolic calcium content. Seven studies examined the effect of omega-3 fatty acids on cytosolic calcium content. One study directly compared the effect of acute and chronic exposure to free DHA on cytosolic calcium content[91]. This study showed that both acute and chronic exposure to DHA were effective in decreasing the magnitude of increase in cytosolic calcium content induced by an agent (potassium chloride [KCl]) or under an anoxic condition. See Table 3-28.

While 2 of the studies [74,90] showed that free EPA decreased cytosolic calcium content, the other 4 studies showed that neither free nor bound EPA or DHA had an effect on cytosolic calcium content [61,72] {Vitelli, 2002 100059 /id}[78]. In the presence of various agents (NIT, BAY, ISO, KCl, Endothelin-1, Ca^{2+} free Krebs Ringer bicarbonate buffer [KRB], Doxorubicin [DXR] and caffeine), free or bound EPA and DHA blocked the alterations in cytosolic calcium induced by these agents. See Table 3-28.

Sarcoplasmic reticulum calcium content. Only 1 study using a rat model examined the effect of free EPA on sarcoplasmic reticulum calcium content and reported an increase in the presence of caffeine [74]. See Table 3-28.

Sarcoplasmic reticulum calcium uptake. No studies were identified that specifically studied the effect of omega-3 fatty acids on sarcoplasmic reticulum uptake of calcium. See Table 3-28.

Sarcoplasmic reticulum calcium release. Two studies examined the effect of omega-3 fatty acids on sarcoplasmic reticulum calcium release. One of these studies used both rat and guinea pig models and found that free EPA significantly decreased the sarcoplasmic reticulum calcium release [77]. Another study using a rat model found that free DHA increased sarcoplasmic reticulum calcium release in the presence of DXR and caffeine [92]. See Table 3-28.

Sodium-calcium and sodium-hydrogen exchangers. There were 3 studies that examined the effect of omega-3 fatty acids on sodium-calcium and sodium-hydrogen exchange. Two of these studies used a canine model and were by the same investigator. Both reported that free ALA increased sodium-calcium exchange. The other study used a pig model and showed that free ALA did not affect sodium-hydrogen exchange [93,94]. However, there was a dose-dependent decrease attributable to EPA at 50 and 100µM, but not at 10 and 25µM. DHA also decreased the sodium-hydrogen exchange. See Table 3-28.

Other ion pump and ion movement outcomes. One study using a rat model showed that free EPA decreased calcium transients [95]. Two studies by the same investigator using canine models

showed a significant increase in passive sarcoplasmic reticulum calcium efflux attributable to free ALA [93,94]. One study using a pig model showed that free EPA or free DHA had no impact on passive sodium influx [96]. See Table 3-28.

Ion Currents

This section describes 12 studies that examined the effect of free omega-3 fatty acids on ion currents in isolated organs or cells; 1 study used a mouse model, 7 of the studies used rat models; 1 used a guinea pig model, 1 used a ferret model, and 2 used both rat and guinea pig models. See Table 3-29.

Sodium current. Three studies examined the effect of free omega-3 fatty acids on sodium current parameters (I_{Na}) including amplitude, the current-voltage relation, and activation and inactivation parameters. The first study, using a rat model, demonstrated a significant shift to more positive potentials in the voltage dependence of activation, and a significant shift to more negative potentials in the inactivation of the sodium current, using ALA, EPA, or DHA [97]. A study using both rat and guinea pig models found a dose-dependent decrease in peak amplitude of the sodium current with both EPA and DHA [83]. In another study using a rat model, a significant time, dose, and voltage-dependent decrease of the sodium current was observed using ALA, EPA, or DHA. There was, however, no change in the current-voltage relationship and activation or inactivation parameters of the sodium current [98]. See Table 3-29.

Transient potassium outward current. Four studies examined the effect of free omega-3 fatty acids on transient potassium outward current (I_{to}) parameters, including amplitude, frequency, and the time constant of transient potassium outward current. The first of these studies used a rat model and showed that both EPA and DHA decreased I_{to} amplitude and the time constant of I_{to} inactivation, and increased the I_{to} delay [88]. The presence of indomethacin did not modify this effect, suggesting that the effects of the omega-3 fatty acids are not related to their cyclo-oxygenase products. In the second of these studies using a rat model, there was a dose dependent decrease in I_{to}[83] . In the third study, which also used a rat model, EPA significantly decreased the frequency and significantly increased the amplitude of I_{to} [90]. In the last study, which used ferrets, ALA, EPA, or DHA significantly decreased I_{to} amplitude (ALA<EPA<DHA) [99]. See Table 3-29.

Voltage dependent L-type calcium current. Six studies examined the effects of free omega-3 fatty acids on the voltage dependent L-type calcium I_{Ca}.L currents. Using a rat and guinea pig model, one study found there was a dose-dependent decrease in voltage dependent L-type calcium current with both EPA and DHA [83]. Similarly, in a rat and guinea pig model study comparing EPA to standard chow, there was a significant decrease in voltage dependent L-type calcium current [77]. In a rat model study, both EPA and DHA decreased the amplitude of voltage dependent L-type calcium current [74]. In a study examining the effect of various agents on voltage dependent L-type calcium current, DHA increased the amplitude of the current in the presence of nitrendipine. DHA also blocked the BAY K8644-induced increase in voltage dependent L-type calcium current amplitude, but did not change the amplitude in the presence of isoproteronol or in the absence of an agent [61]. In another study using a rat model, significant time, dose, and voltage-dependent decreases in voltage dependent L-type calcium current were observed in the

presence of ALA, EPA, or DHA, along with a negative shift in the voltage dependent L-type calcium current inactivation curve [95]. In a study of guinea pigs using methylated DHA, a significant increase in voltage dependent L-type calcium current was observed [82]. See Table 3-29.

Delayed rectifier potassium current. Two studies examined the effect of free omega-3 fatty acids on delayed rectifier potassium current (I_K). One study observed a decrease in I_K using EPA in both rat and guinea pig models [83], and the other study, using a ferret model, also showed a significant decrease with either ALA, EPA, or DHA [99]. See Table 3-29.

Inward rectifier potassium current. Four studies examined the effect of free omega-3 fatty acids on inward rectifier potassium current (I_{KI}). One study using a mouse model showed no effect of DHA [100]. Another, using a rat model, showed no effect of either EPA or DHA [88]. A third study using EPA with rat and guinea pig models showed a decrease in rectifier potassium current [83]. The ferret model study showed no change using ALA, EPA, or DHA [99]. See Table 3-29.

Ultra rapid potassium current. Two studies examined the effect of free omega-3 fatty acids on ultra rapid potassium current (I_{KUR}). One using a mouse model showed a significant decrease in I_{KUR} with 30µM of DHA[100]. The other study, using a rat model, showed a significant decrease in I_{KUR} at dosages above 20µM of EPA or DHA but no effect with an EPA dose of 5-10µM [88]. See Table 3-29.

Ion Channels

Three studies examined the effect of omega-3 fatty acids on ion channels in isolated organs or cells; 1 study used a mouse model [100] and 2 used rat [65,101]. In the mouse model study, the investigators examined the effect of free DHA on activity of the cloned Kv1.5 potassium channel, and observed that while DHA significantly blocked this activity, free ALA had no effect. See Table 3-30.

One of the rat model studies examined nitrendipine binding to putative dihydropyridine-sensitive calcium channels and reported that both bound EPA and bound DHA significantly decreased both the high and low affinity binding sites (B_{max}) as well as the K_d values of those binding sites. With DHA, the high affinities were so diminished that they were undetectable [65]. The other study using a rat model examined the effect of bound EPA on the number of sodium channels per cell and showed no change; however, the combination of EPA with mexiletine significantly reduced the number of sodium channels and blocked the mexiletine-induced increase in sodium channel expression [101]. See Table 3-30.