The undersigned, appointed by the dean of the Graduate School,

have examined the thesis entitled

PARENTERAL FISH OIL EFFECTS ON PLASMA NON-ESTERIFIED FATTY ACIDS AND SYSTEMIC CYTOKINE CONCENTRATIONS IN DOGS IMMEDIATELY FOLLOWING OVARIOHYSTERECTOMY

presented by Kaoru Tsuruta,

a candidate for the degree of

Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

F. A. Mann, DVM, MS, Diplomate ACVS, Diplomate ACVECC

R. C. Backus, MS, DVM, PhD, Diplomate ACVN

Amy DeClue, DVM, MS, Diplomate ACVIM (SAIM)

Kevin Fritsche, PhD
DEDICATION

This work is dedicated to my parents, my brother, my friends, and ex-coworkers in both Japan and the USA.

Thank you for encouraging me all the time. Your warm messages and gifts from Japan helped me to keep my enthusiasm and hope. Your advice taught me how to get over difficulties and live comfortably in a different country.

I wish all of you happiness and peace.
ACKNOWLEDGEMENTS

I would like to acknowledge Dr. F. A. Mann for his advice and support during my entire program. I appreciate the opportunity to come and study here in the USA. Dr. Mann had the patience and appropriate advice whenever it was needed. He also gave me many opportunities to participate in clinical courses and projects. Those helped me to broaden my knowledge. I am indeed fortunate to have him as a supervisor.

I would also like to acknowledge Dr. R. C. Backus, who taught me basic concepts of research as well as techniques. By his teaching philosophy, I learned to be a graduate student. I appreciate his patience with me and dedication to the project. I am inspired by his enthusiasm for research and dedication to graduate training.

I would also like to acknowledge the other members of my committee, Dr. Amy DeClue and Dr. Kevin Fritsche. I appreciate their technical support and teaching. Their dedication to the cause of this study helped me to accomplish the major details of the experiments.

I would also like to acknowledge Dr. Jimmy Browning and Dr. Aaron Stoker for providing technical support.

Finally, I would like to acknowledge Ms. Shelly Hoffman for her kindness and support for this project. Without her management, I am sure that this project would not have been accomplished smoothly.
TABLE OF CONTENTS

ACKNOWLEDGEMENT.................................................................ii

LIST OF ILLUSTRATIONS..........................................................v

LIST OF ABBREVIATIONS...........................................................vii

ABSTRACT................................................................................ix

Chapter

1. INTRODUCTION.................................................................1

   Fatty acids
   Omega-3 fatty acids and omega-6 fatty acids
   Parenteral nutrition
   Lipid metabolism in critically illness
   The effects of omega-3 fatty acids on inflammation and immunity
   Oral and intravenous supplement of omega-3 fatty acids
   The objective and hypothesis of the study

2. MATERIALS AND METHODS..............................................11

   Subjects
   Surgery and anesthesia protocol
   Intervention
   Blood samples
   Assays
      Total non-esterified fatty acids concentration measurement
      Fatty acids analysis of red blood cells
      Non-esterified fatty acids analysis in plasma
      Leukocyte cytokine production capacity assay
      Cytokine concentration measurement
      Statistical analysis

iii
3. RESULTS..................................................................................................................23

    Study population
    Total non-esterified fatty acids concentrations in plasma
    Fatty acids profile on red blood cell membranes
    Non-esterified fatty acids profile in plasma
    Leukocyte cytokine production capacity assay

4. DISCUSSION............................................................................................................31

5. FUTURE DIRECTIVES..............................................................................................44

APPENDIX

1. Tables......................................................................................................................46

2. Figures......................................................................................................................51
LIST OF ILLUSTRATIONS

Table
1. Composition of the lipid emulsions……………………………………46
2. Signalments of dogs in each treatment group………………………….47
3. Experimental design and blood collection schedule…………………...48
4a. Plasma non-esterified fatty acids composition of treatment groups.………59
4b. Plasma non-esterified fatty acids expressed in concentrations units…………50

Figures
1. Chemical structures of omega-3 and omega-6 fatty acids………………51
2. Metabolism of omega-6 and omega-3 fatty acids……………………52
3. Omega-3 fatty acids inhibit the nuclear factor-kappa B signaling pathway….53
4. Comparison of total non-esterified fatty acids concentration in infusion
groups by enzymatic and gas-chromatography analyses……………………54
5. Correlation of total non-esterified fatty acids concentrations between enzymatic
and gas-chromatography analyses…………………………………………55
6. Fatty acids concentrations in erythrocytes……………………………..56
7. Arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid and
total long-chain omega-3 fatty acid concentrations in plasma………………57
8a. Comparison of interleukin-6 production from whole blood culture
    after stimulation with PBS, LPS, LTA and PG in the three treatment groups…60
8b. Comparison of interleukin-10 production from whole blood culture
    after stimulation with PBS, LPS, LTA and PG in the three treatment groups…62
8c. Comparison of tumor necrosis factor-alpha production from whole blood culture after stimulation with PBS, LPS, LTA and PG in the three treatment groups…….64
LIST OF ABBREVIATIONS

AA  Arachidonic acid
AAFCO Association of American feed control officials
ACOD acyl-CoA oxydase
ACS acyl-CoA synthetase
ALA $\alpha$-linolenic acid
AUC Area-under-the curve
BCS Body condition score
BW Body weight
COX Cyclooxygenase
CV Coefficient variation
DHA Docosahexaenoic acid
DPA Docosapentaenoic acid
EDTA ethylenediaminetetraacetic acid
EN Enteral nutrition
EPA Eicosapentaenoic acid
FA Fatty acids
GC Gas-chromatography
HCL Hydrochloric acid
HDL High-density lipoprotein
HPLC High-performance liquid chromatography
HSL Hormone sensitive lipase
IKK I KappaB kinase
IL-6 Interleukin-6
IL-10 Interleukin-10
IM intramuscular
IV intravenous
LA Linoleic acid
LOX 5-Lipoxygenase
LPL Lipoprotein lipase
LPS Lipopolysaccharide
LT Leukotriene
LTA Lipotechoic acid
NEFA Non-esterified fatty acids
NF-kB Nuclear factor-kappa B
NSAID non-steroidal anti-inflammatory drugs
OVH Ovariohysterectomy
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAMP</td>
<td>Pathogen-associated molecular pattern</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PG</td>
<td>Peptidoglycan</td>
</tr>
<tr>
<td>PGI₃</td>
<td>Prostaglandin I₃</td>
</tr>
<tr>
<td>PLA₂</td>
<td>Phospholipase A₂</td>
</tr>
<tr>
<td>PN</td>
<td>Parenteral nutrition</td>
</tr>
<tr>
<td>PO</td>
<td>per oral</td>
</tr>
<tr>
<td>POD</td>
<td>peroxidase</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>sPLA₂</td>
<td>Secreted phospholipase A₂</td>
</tr>
<tr>
<td>TG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumor necrosis factor-alpha</td>
</tr>
<tr>
<td>Total LC omega-3</td>
<td>Total Long-chain omega-3 fatty acid</td>
</tr>
<tr>
<td>TX₂</td>
<td>Thromboxan A₂</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
</tbody>
</table>
ABSTRACT

An extreme systemic inflammatory response may be observed in dogs that experience severe accidental trauma, such as being struck by a car. The inflammatory response may be so extreme that it complicates treatment and causes multiple organ failure. There are few currently available methods to counter an extreme systemic inflammatory response. The aim of this project was to use an ovariohysterectomy model to determine if systemic inflammatory mediators released in response to controlled trauma can be modulated by a fish oil infusion high in omega-3 fatty acids.

Three different cephalic vein infusates (fish oil, soybean oil, or saline) were given to dogs immediately following ovariohysterectomy and cytokine concentrations in jugular venous plasma samples were compared. After fish oil infusion, plasma omega-3 fatty acid concentration markedly increased, but the increased concentration was not long-lasting. Cytokine production when stimulated by pathogen-associated molecular patterns was increased by trauma (ovariohysterectomy), but it was not suppressed by fish oil infusion. Assuming cytokine production by circulating
leukocytes is a marker of systemic inflammation, fish oil emulsion infusion at the
dose and duration studied did not appear to attenuate the systemic inflammatory
response.
Chapter 1

Introduction

Fatty acids

Fatty acids (FA) consist of hydrocarbon chains with a carboxylic acid group (COOH) on one terminus and a methyl group (CH₃) on the opposite terminus. Fatty acids with no double bonds in the hydrocarbon chain are called saturated, whereas those with one double bond are monounsaturated and those with more than one double bond are polyunsaturated fatty acids (PUFA).¹ ² Fatty acids are often described as A:Bn-C. The “A” represents the number of carbon atoms in the fatty acid and the “B” is the number of double bonds in the fatty acid. The “C” is the order of carbon chain counting from the terminal methyl end in which a double bond is located.

Saturated fatty acids can be synthesized endogenously de novo or derived from the diet. Subsequent endogenous alteration of saturated fatty acids occurs through desaturation via enzymatic activity, resulting in the formation of unsaturated fatty acids.

Plasma non-esterified fatty acids (NEFA) are fatty acids that are not bound in the form of esters of glycerol, cholesterol, or other substances. These NEFA ionize at plasma pH but mostly bind when formed immediately with albumin, and are
transported in blood to the tissues where they may be used in various ways, such as, to provide energy. In adipose tissue, FA are esterified with glycerol and are stored principally in triacylglycerol (TG). Fatty acids are provided by diet and de novo synthesis and used as the main source of metabolic fuel during a normal fasting period. Within the cell, FA serve a variety of roles, including: (1) incorporation into cell membrane phospholipids where their saturation affects the physical properties of the membrane and membrane-associated protein function, (2) incorporation into TG to be stored in adipose tissue or assembled to circulate in blood as lipoproteins after processing in liver, and (3) utilization as precursors for synthesis of lipid mediators such as prostaglandins, leukotrienes (LTs), protectins, and resolvins.

**Omega-3 fatty acids and omega-6 fatty acids**

Omega-3 FA, which are commonly labeled ω-3 FA or n-3 FA, have the first double bond between the third and fourth carbon counting from the methyl end. On the other hand, omega-6 FA, which are commonly labeled ω-6 FA or n-6 FA, have the first double bond between the sixth and seventh carbon (Figure 1).

Animals can synthesize saturated fatty FA de novo from glucose and amino acids, and these ultimately may be unsaturated to 16- and 18-carbon monounsaturated FA becoming omega-7 and omega-9 FA. Other fatty acid types cannot be synthesized by animals. Unlike plants, animals lack enzymes (desaturases) that insert additional
double bonds toward the methyl terminus in omega-9 FA (18:1n-9). Therefore, animals cannot synthesize the parent fatty acid of omega-6 and omega-3 FA families, such as linoleic acid (18:2n-6) and α-linolenic (18:3n-3) acid.12 Both omega-3 and omega-6 FA are considered essential for dogs and cats because these FA cannot be synthesized de novo, and are required for normal metabolism, growth, reproduction and other processes necessary for like. In humans, α-linolenic acid (18:3n-3) and linolenic acid (18:2n-6) are considered “essential fatty acids”.6 Linoleic acid is regarded as a dietary essential fatty acid in dogs and other species. Arachidonic acid (AA, 20:4n-6) is also considered an “essential fatty acid” in cats. Cats have limited enzyme activities (Δ6 and Δ5 desaturases) required for synthesis of AA from linoleic acid. Therefore, AA is also nutritionally essential for cats.1 In humans and dogs, AA is synthesized from linoleic acid and is essential for tissue growth.

Eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) may be synthesized in animals from α-linolenic acid, and may modify inflammation and immune responses. These long-chain omega-3 PUFA are also essential for the structure of the retina and brain.

The common dietary sources of omega-3 FA are fish oil and some plants such as flaxseed. Dietary sources of omega-6 FA are vegetable oils, soybeans, and eggs. Omega-3 FA are reported to be effective for treatment of some skin disorders.7
In addition, long-chain omega-3 fatty acid derivatives of α-linoleic acid such as DHA are reported to distribute to the brain and retinal rod cells in dogs. Docosahexaenoic acid is demonstrated to be essential for cognitive and visual development. However, the conversion of α-linoleic acid to DHA is limited. Therefore, dog foods are recommended to contain the long-chain omega-3 FA, EPA and DHA as well as α-linoleic acid.

Dietary omega-3 FA are well known to reduce circulating plasma TG concentration by decreasing production of very low density lipoprotein (VLDL) and increasing in TG clearance. In addition, dietary supplementation of omega-3 FA was reported to modulate immune responses and play a preventive role as well as a treatment for cardiovascular disease, arthritis, atopic dermatitis, inflammatory bowel disease, cancer, surgical trauma, and acute lung injury in people, dogs, and cats.

**Parenteral nutrition**

As negative energy balance in critically ill patients is associated with serious complications, such as sepsis, nutritional support is a crucial part of the treatment regimen of critically ill patients. To supply nutrients to critically ill patients who cannot intake sufficient calories voluntarily, assisted enteral (EN) and parenteral nutrition (PN) are used. With assisted EN, food is typically instilled via a feeding tube
into the gastrointestinal tract, whereas, with PN, sustenance is provided as intravenous nutrients. For animals that cannot tolerate enteral feeding, PN feeding is used, and can be administered via a central or peripheral vein.

Parenteral admixtures commonly used in veterinary PN are simple. Most are composed of solutions of amino acids, dextrose, and B-vitamins with variable addition of a lipid emulsion. Currently, many kinds of parenteral lipid emulsions are commercially available. These lipid emulsions have similar osmolarity to peripheral blood and contain biologically active compounds such as essential FA. Conventional types of lipid emulsions contain soybean oil as the majority lipid. The major PUFA component of soybean oil is omega-6 fatty acid (linoleic acid, 18:2n-6). On the other hand, lipid emulsions based on fish oil, which contain omega-3 FA, are becoming available (Table 1). Given their putative effects on modulating the inflammatory response, intravenous administration of fish oil-containing lipid emulsions is investigated as potential adjunctive treatment in critically ill patients.25-33

**Lipid metabolism in critically illness**

A large mass of FA is stored as TG in adipose tissue. The rate of fatty acid deposition and release by adipose tissue is regulated by enzymes in response to nutritional condition and hormone activity. Hormone sensitive lipase (HSL) exists in
adipose tissue and hydrolyzes TG releasing NEFA and glycerol which may enter into the blood. During fasting, plasma insulin concentration falls and glucagon concentration rises, leading to increases of HSL activity and resulting in increases of TG hydrolysis and net efflux of NEFA into circulating blood. The NEFA released from adipose tissue is transported to tissues where it may be used as an energy source. A minor fraction of NEFA may be taken up by the liver and re-esterified to glycerol to make TG, and be assembled into lipoproteins that enter plasma. On the other hand, adipose tissue lipoprotein lipase (LPL) that is attached to the inner surface of endothelial cells of capillaries is deceased in activity during fasting. Low LPL activity results in diminished intravascular lipolysis of TG and reduced re-esterification of NEFA within adipose tissue. A phenomenon affecting NEFA release and uptake by adipose that is somewhat similar to fasting occurs during surgical injury and other physical stresses. During injury, anti-insulin hormones such as cortisol, glucagon and catecholamines (i.e., epinephrine and norepinephrine) are elevated. Increased epinephrine and glucagon increase HSL activity, leading to intra-adipocyte lipolysis and release of NEFA, the preferentially utilized fuel in the stress situation. Tumor necrosis factor-alpha (TNFα) that is released by macrophages in critically ill animals also can activate HSL and another type of lipase, such as adipose triacylglycerol lipase, and induce lipolysis.
Omega-3 and omega-6 FA are important constituents of immune cell membranes. Eicosanoids derived from these FA, such as AA, are mediators in regulation of phagocytosis and T and B lymphocyte functions. In immune cells that mediate inflammation, such as macrophages, omega-3 and omega-6 FA in membranes are released by the action of phospholipase A2 (PLA2). Phospholipase A2 is activated by a variety of inflammatory stimuli, including TNFα. Once omega-3 and omega-6 FA are released from membrane phospholipids, they can be metabolized into a wide variety of lipid mediators (described below).

The effects of omega-3 fatty acids on inflammation and immunity

Although inflammation is a normal host defense response to trauma or infection, an extreme and uncontrolled inflammatory response may lead to excessive tissue damage and worsening illness, possibly leading to multiple organ failure and death. Omega-3 FA are reported to have anti-inflammatory effects through several mechanisms (Figure 2). Firstly, omega-3 FA are reported to inhibit activation of the nuclear factor-kappa B (NF-kB), which acts as a crucial transcription factor involved in the production of proinflammatory cytokines, chemokines, and adhesion molecules. Omega-3 FA can block the binding of lipopolysaccharide (LPS) to toll-like receptors, a gram-negative bacterial membrane constituent. Omega-3 FA also prevent
I Kappa B kinase (IKK) phosphorylation in the canonical NF-kB pathway. In addition, omega-3 FA can act as agonists of peroxisome proliferator-activated receptor (PPAR) that induces lipogenesis in adipose tissue in response to hormones such as catecholamine and glucocorticoids. When omega-3 FA bind to PPAR, PPAR is activated and interacts directly with NF-kB to interfere with its nuclear translocation and DNA binding activity.\textsuperscript{4,46-47} Secondly, omega-3 FA can be metabolized to less inflammatory eicosanoids, such as 3 series of prostaglandins (e.g. prostaglandine I\textsubscript{3}, [PGI\textsubscript{3}]) and 5 series of leukotrienes (e.g., leukotriene B\textsubscript{5}, [LTB\textsubscript{5}]) by cyclooxygenase (COX) and 5-lipoxygenase (LOX), and, as a consequence, pro-inflammatory AA derived eicosanoids such as 2 series of prostaglandine (e.g., PGE\textsubscript{2}) and 4 series of LT (e.g., LTB\textsubscript{4}) are decreased due to substrate restriction (Figure 1).\textsuperscript{42,48} Lastly, lipid mediators derived from EPA and DHA called resolvins, protectins, and maresins play a role in resolution of inflammation.\textsuperscript{49} Therefore, by these mechanisms, omega-3 fatty acid administration to a patient could change the component of FA in immune cell membrane, and thereby alter inflammation and immune function.

\textit{Oral and intravenous supplement of omega-3 fatty fatty acids}

Cold-water, marine fish oils are especially enriched in the long-chain omega-3 FA, EPA and DHA. Dietary supplementation with these fish oils is reported
to be effective in the modulation of inflammatory mediators in response to endotoxin in healthy dogs.\textsuperscript{20} The effects of the supplementation were determined after three months of intake of fish oil-enriched diet containing 1.75 g of EPA/kg of diet and 2.2 g of DHA/kg of diet (dry-matter basis). Also, cold-water marine fish oil has been used to prevent inflammation caused by surgical trauma. In a study with humans undergoing major surgery, omega-3 fatty acid supplementation was suggested at least five days prior to surgery to enhance immunity.\textsuperscript{50} In other studies, dogs with osteoarthritis had improved arthritic condition after 6 months eating of a diet containing 3.5 % omega-3 FA (dry-matter basis).\textsuperscript{19,20} In a study conducted in healthy people, modulation of immune response was observed within only one week of fish oil supplementation.\textsuperscript{51}

In a critical care setting, a rapid onset of modulating inflammation is needed. Parenteral administration of fish oil has been reported to modulate the inflammatory response.\textsuperscript{52} In a previous study of healthy human subjects, intravenous administration of fish oil achieved rapid EPA incorporation in platelet phospholipids and showed biological effects in a few hours.\textsuperscript{30} Omega-3 fish oil could theoretically cause a bleeding disorder because of effects of reducing production of AA metabolites, thromboxane A\textsubscript{2} (TXA\textsubscript{2}) and platelet activating factor.\textsuperscript{53,54} However, no adverse effects are reported with the parenteral administration of fish oil lipid emulsion with a
maximum recommended dosage of 0.2 g/kg of body weight (BW) in people.\textsuperscript{25,55}

These findings on parenteral administration indicate potential beneficial use for fish oil administration in critically ill animals.

\textit{The objective and hypothesis of the study}

The objective of this study was to determine if inflammatory mediators released in response to surgical trauma (routine ovariohysterectomy [OVH] surgery) in dogs are modulated by short-term intravenous administration of fish oil emulsion after surgery. As far as we know, this is the first report of the effect of administration of fish oil emulsion in dogs after a surgical trauma. It was hypothesized that: (1) total NEFA concentration of plasma would increase after surgical trauma, (2) omega-3 FA concentration in plasma would increase after fish oil emulsion infusion, and (3) the release of inflammatory mediators from cultured blood cells, such as TNF\(\alpha\), interleukin-6 (IL-6), interleukin-10 (IL-10), would be attenuated by infusion of fish oil emulsion.
Chapter 2

Materials and Methods

Subjects

All dogs presented to the University of Missouri Veterinary Medical Teaching Hospital for elective OVH between August 2011 and April 2012 were eligible for enrollment if they met criteria for inclusion. Enrollment was continued until thirty dogs meeting the inclusion criteria were identified and completed the study protocol. The dogs were randomly assigned to three groups (n=10 for each group) that received the same care, but differed only in type of parenteral infusion given after surgery. The inclusion criteria were: 4 months of age or older; not currently showing signs of estrus (“heat”) or known to be pregnant; not receiving non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or heparin-containing agents; not known to be intolerant of fish, soybean or egg; appearing healthy and in appropriate nutritional condition based on physical examination; and weighing at least 4 kg.

After enrollment of the initial 4 dogs, a maximum body weight limit of 20 kg was set. The maximum body weight limit ensured that enough of the same lot of fish oil emulsion would be available for infusions in 10 dogs. For dogs found to be in heat, enrollment was rescheduled at least one month after termination of estrus. If dogs
were administered vasopressor agents during surgery, such as ephedrine, they were excluded from the study due to possible effects of modulation of fat metabolism by the agents.

Owners of potential study subjects were interviewed, supplied written description of the study protocol, and signed an informed consent form. The study procedures, consent form, and experimental protocol were approved by the Animal Care and Use Committee of the University of Missouri.

_Surgery and anesthesia protocol_

Ovariohysterectomy was performed by veterinary students under the supervision of a licensed veterinarian. A standardized OVH procedure and anesthetic protocol was used for all dogs. Premedication drugs were dexmedetomidine (5 µg/kg IM) and morphine (0.5 mg/kg IM). For induction, propofol was given IV to effect, up to 6 mg/kg. During surgery, anesthesia was maintained with isoflurane in inhaled oxygen. Postoperative analgesia drugs were dexmedetomidine (2 µg/kg IM), and morphine (0.5 mg/kg IM). During the immediate postoperative recovery period, dexmedetomidine (2 µg/kg IM) and morphine (0.5 mg/kg IM) were administered. Lactated Ringers solution was infused IV at a rate of 10 ml/kg/hr for the first hour of anesthesia, and then continued at 5 ml/kg/hr until the end of anesthesia. If a dog was
found to be pregnant during surgery, or an alternative analgesia protocol was used according to the attending clinician’s discretion, the dog was excluded from the study.

**Intervention**

Thirty dogs meeting inclusion criteria were assigned to one of three groups (10 dogs per group) according to type of postoperative intravenous infusion (Table 2): Group 1 received fish oil emulsion (Omegaven 10%, 10G Fish oil; Fresenius KABI Deutschland GmbH, Germany); Group 2 received soybean oil emulsion (Intralipid 10%, Baxter US, U.S.A.); and Group 3 received sterile saline (sodium chloride 0.9%, Hospira Inc. Lake forest, IL, USA). For Group 2 infusions, 20% Intralipid was diluted 1:1 with 0.9% saline to provide a solution of the same lipid concentration as Omegaven. The same volume (2 ml/kg BW) of postoperative infusion was given for 3 hours to each dog in each group.

The time of post-surgical extubation was recorded, and 1.5 hours later test solution infusion was started via the cephalic venous catheter that was inserted prior to surgery before anesthetic induction. Neither heparin nor any other anticoagulant was placed in the catheter. The duration of treatment infusion was three hours. After a 30-minute washout period following the infusion, a jugular venous blood sample (10 ml) was drawn by venipuncture and the cephalic venous catheter was removed. The
dogs were then presented the same commercial food (Purina Proplan Chicken and Rice, Nestle Purina Pet Care, St. Louis, MO, USA) in an amount calculated to meet half of their resting energy requirement (RER; $70 \times \text{BW}^{0.75}$). The food was withdrawn 12 hours before the next day’s blood collection. Free-choice water was supplied overnight. An analgesic agent (tramadol hydrochloride, 1 to 4 mg/kg PO) was given at the time of food presentation. The analgesic agent was given again on the next day after the last blood collection, but with the dose and subsequent frequency determined by the attending clinician.

**Blood samples**

A series of three blood samples (10 ml) were collected via jugular vein from each dog (Table 3). On admission day, dogs were hospitalized overnight to ensure 12 hours of food withholding before the planned surgery. On the next day, a “pre-surgery” blood sample was drawn before premedication for anesthesia. A “5 hours post-surgery” blood sample was collected after a 30-minute washout period following infusion. On the day after surgery (24 hours after the dog was extubated), a “24 hours post-surgery” blood sample was collected.

Collected blood was aliquoted into tubes containing either potassium EDTA (0.06 ml of 7.5% EDTA ($K_3$)-containing tube, Tyco Healthcare Group LP, Mansfield,
MA, USA) or sodium heparin (BD Vacutainer® Sodium heparin^N 158 USP units, BD Franklin, Lakes, NJ, USA). Immediately after collection, EDTA anti-coagulated blood was centrifuged at 1500×g at 4 °C for 7 minutes. Then, the plasma was separated and stored at -80 °C for total and individual NEFA analyses. Red blood cells (RBCs) were retained after plasma was separated and stored at -80 °C for the fatty acid profile of their membranes. Sodium heparin anticoagulated blood was used to determination leukocyte cytokine production capacity (see “Leukocyte cytokine production capacity assay” for details).

**Assays**

*Total non-esterified fatty acids concentration measurement* - Plasma total NEFA concentration was determined with a commercially available kit according to manufacture directions but modified as appropriate for use on 96-well plates (HR Series NEFA-HR (2) kit, Wako chemicals, VA, USA). The assay is an enzymatic method that utilizes acyl-CoA synthetase (ACS), acyl-CoA oxydase (ACOD) and peroxidase (POD). Briefly, plasma samples (10 µL) were mixed with ACS and coenzyme A (CoA) containing solution (400 µL) at ambient temperature (21-22 °C). A portion of mixture (140 µL) was added to wells of the 96-well plate and incubated at 37 °C for 5 minutes so that acyl-CoA esters of NEFA were formed. The acyl-CoA
produced from the FA was oxidized by addition of ACOD containing solution (70 µL) and 5 minutes of incubation at 37 ºC. Produced hydrogen peroxide in the presence of POD formed a purple colored end product. Absorption measured at 550 nm was proportional to concentration of oleic acid standard provided with the kit from 0.125 to 2.5 mEq/L. Sample NEFA concentrations were determined in triplicate with all sampling times (pre-surgery, 5 hours post-surgery and 24 hours post-surgery) with any given dog analyzed on the same plate within the same assay. Observations on serum controls of two NEFA concentrations provided with the kit’s manufacturer reported the intra- and inter-assay coefficients of variation to be 3.1 and 3.3%, respectively.

**Fatty acids analysis of red blood cells** - Eicosapentaenoic acid, DHA and AA concentrations in erythrocytes of pre-surgery blood samples were measured by high-performance liquid chromatography (HPLC) as previously reported. Briefly, washed erythrocytes were re-suspended in an equal volume of 0.9% saline and the resulting number of RBC counted in the saline solution. Aliquots of 400 µl of the erythrocytes suspension were transferred to screw-top tubes containing 100 µl of butylated hydroxytoluene as an antioxidant agent. Margaric acid (17:0) was added as an internal standard and the mixture was heated in methanol:HCL, 5:1 (by vol) for 2.5 hours. The lipids were extracted into 4 ml of hexane:amylalcohol, 40:1 (by vol). The
extracts were evaporated under nitrogen and reconstituted with acetonitrile, maintained under an atmosphere of nitrogen and stored at -20 °C until HPLC analysis.

Fatty acids extracts were derivatized with 4-bromomethyl-7-methoxycoumarin and injected onto a HPLC column that was heated or kept at ambient temperature (21-22 °C) as described below. The mean absolute recovery rate of AA was previously reported to be 17.0% and relative recovery rate of that was 91.0%, whereas those of EPA and DHA were not reported. In this study, absolute and relative recoveries of EPA, AA and DHA were determined according to the previous report.

**HPLC system**: The chromatographic system consisted of a Gilson 234 Autoinjector, 306 Pump and 305 Pump, 805 Manometric Module, 811C Dynamic Mixer (Gilson, Middleton, WI, USA), an Ultrasphere ODS (C18), 5 µ, 2.0 mm × 25 cm column (Beckman Instruments, SanRamon, CA, USA) with an upstream 2 µm stainless steel filter (Up Church Scientific, WA, USA) and guard column (Opti-guard, C18, 1 mm, Optimize Technologies, Oregon City, OR, USA). Fluorescent derivatives of FA were detected with a RF-535 fluorescence HPLC monitor, excitation 325 and emission 395 (Shimadzu, Tokyo, Japan). For one of the two HPLC methods used (the “heating method”), the column was heated with a CH-30\TC-50 column heater\controller (Eppendorf, Haupauge, NY, USA). SRI Model 203 Peak Simple Chromatography Data System Single Channel Serial Port module and software (SRI...
Instruments, Inc., Las Vegas, NV, USA) was used to analyze recorded peaks.

**Heating method:** This protocol was used to evaluate erythrocyte EPA and DHA concentration. The column was heated to 45 °C and mobile phase flow was 0.6 ml/min. Eluents A and B used for a binary gradient were acetonitrile:water (75:25 by vol) and 100% acetonitrile, respectively. The gradient consisted of a linear increase of 0-100% B in 15 minutes. Area-under-the-curve (AUC) of EPA and DHA peaks were recorded and the concentration of each fatty acid was determined by the ratio of each AUC to that of internal standard (margaric acid, 17:0) and adjustment for relative fluorescence of the fatty acid.

**Ambient method:** Because AA peak could not be clearly separated from the palmitoleic acid (16:1) peak by the heating method, a second protocol (the “ambient method”) was used to separate these two peaks and thereby quantify AA. Lauric acid (12:0), a second internal standard was added to aliquots of the FA extract before derivatization. Lauric but not margaric acid was clearly resolved from AA by the ambient method. Column temperature was kept at room temperature (21-22 °C) and mobile phase flow decreased to 0.3 ml/min. Eluents A and B were acetonitrile:water, (30:70 by vol) and 100% acetonitrile, respectively. The gradient consisted of a linear increase of 0-100% acetonitrile in 20 minutes. Area-under-the-curve was recorded and the concentration of AA was determined from the ratio of AUC of AA to that of lauric
acid (12:0) and adjustment for relative fluorescence of the fatty acid.

*Non-esterified fatty acids analysis in plasma* - Non-esterified fatty acids profiles in plasma were determined using gas chromatography. The lipid from plasma samples was extracted with isopropanol/n-heptane/phosphoric acid (2 mol/L; Dole’s mixture, 40/10/1, by vol) using a before described one-step Dole extraction. Non-esterified FA were separated from the lipid extract according to previous report. Briefly, lipid extract was reduced to dryness with nitrogen gas, reconstituted with chloroform (300 µL), applied to a glass column packed with solvent-conditioned Chromabond NH₂ (Macherey-Nagel, Düren, Germany), and washed with 2 to 3 ml of chloroform/isopropanol (2:1, by vol). Using 2 to 3 ml of diethyl ether/acetic acid (98:2, by vol) as a solvent, the NEFA were eluted from the column and dried with nitrogen gas. Methyl esters of the NEFA were obtained by reaction with methylalcohol/acetyl chloride (17:1, by vol). The FA methylesters were separated with a gas chromatograph (7890A GC system, Agilent Technologies, Santa Clara, CA, USA) equipped with a 60 m×0.25 mm DB-23 capillary column with 0.15 µm film and 7683B Series injector. Fatty acids peaks were determined by gas chromatography (GC) Chemstation software (B.03.02). Total NEFA concentration was also calculated by summing all FA concentrations detected by GC.
Leukocyte cytokine production capacity assay – Stimulated leukocyte TNFα, IL-6 and IL-10 production of all freshly-collected blood samples were cultured and stimulated as previously described.\textsuperscript{58-64} Briefly, whole blood (4.5 ml) was combined 1:2 with Roswell Park Memorial Institute media (200 U penicillin/ml, 200 μg streptomycin/ml and 200 mM L-glutamine, Corning inc., Corning, NY, USA), the mixture was added to the wells of a 12-well plate. Three types of pathogen-associated molecular patterns (PAMPs), LPS from \textit{Escherichia coli} 0127:B8 (Sigma-Aldrich St. Louis, MO) 100 ng/ml, lipoteichoic acid (LTA) from \textit{Streptococcus aureus} (Sigma-Aldrich, St. Louis, MO) 1000 ng/ml, peptidoglycan (PG) from \textit{Staphylococcus aureus} (Sigma-Aldrich, St. Louis, MO) 1000 ng/ml and phosphate buffered saline (PBS) as a control were added to individual wells. Samples were incubated for 24 hours at 37 °C in a 5% carbon dioxide atmosphere. Plates were then centrifuged (400×g for 7 minutes) and supernatant was collected and stored at -80 °C until later analysis of cytokine concentration.

Cytokine concentration measurement – Tumor necrosis factor-alpha, IL-6 and IL-10 concentrations were measured by MILLIPLEX\textsuperscript{®} MAP Canine Cytokine panel kit (Millipore, Billerica, MA, USA) according to the manufacturer’s instructions. Each sample was analyzed in duplicate. Canine recombinant TNFα, IL-6 and IL-10 were used to construct a standard curve to quantify the concentration of
each cytokine in the test well. Analyses were performed on a Luminex 200, HTS with
xPOTENT software. Assay sensitivity reported for each cytokine assay was 6.1, 3.7,
and 8.5 pg/ml in TNFα, IL-6, and IL-10, respectively. Intra- and inter-assay precision
of all cytokines are <5 and <15 (% CV) respectively.

Statistical analysis

The data were analyzed using the IBM SPSS 20 statistics program (IBM,
Armonk, NY, USA). Statistical comparisons on age, body weight, and body condition
score (BCS) of subjects in each treatment group was made using one way ANOVA or
Kruskal-Wallis test for parametric and nonparametric data, respectively. Generalized
estimating equations were used to evaluate treatment effects (Omegaven, Intralipid
and saline), comparison among time points, and interaction between each time point
and treatment group for total and individual NEFA concentrations, and whole blood
culture leukocyte cytokine production capacity. A one-way ANOVA was used to
compare AA and EPA concentration in erythrocyte membranes among treatment
groups (Omegaven, Intralipid and saline). If data were not normally distributed,
values were transformed by using a natural logarithm or square root function to
address the issue of normality assumption. A least significant difference comparison
procedure or Bonferroni correction was used for post hoc analysis if needed. A p<0.05
was considered statistically significant.
Chapter 3

Results

Study population

Thirty-two dogs initially participated in the study. Two dogs were excluded due to ephedrine administration during surgery. One dog was excluded because its body weight was 26.2 kg, which was far beyond the 20 kg upper limit of body weight. Therefore, a total of 29 dogs were studied. (The number of dogs assigned to each treatment group was the following: Omegaven, n=10; Intralipid, n=10; saline, n=9)

There were a total of 13 breeds represented. The most common breeds were mixed breed (n=14), Boston terrier (3) and Australian shepherd (2). Other breeds were: German short-haired pointer, Jack Russell terrier, Irish terrier, Australian cattle dog, pug, Boykin spaniel, bichon frise, American staffordshire terrier, Alaskan malamute, and shih tzu. The median age was 9 months with a range of 4 months to 5.1 years. The median BW was 12.4 kg with a range of 4.3 to 20.4 kg. The median BCS was 5/9 (Table 2). There was no significant difference in age, BW, and BCS among the treatment groups. Two dogs that were slightly beyond the upper limit of BW (20.2 kg and 20.4 kg) were included in the study.

Information provided by owners in response to questionnaire were: one third of dogs were fed puppy food, no dogs were given fish oil supplement, no dogs were
given medications except heartworm preventive and flea control agents, all owners reported that their dogs had no or unknown allergies against fish, soybean or egg, and no dogs were currently in heat or within one month of estrus.

**Total non-esterified fatty acids concentration in plasma**

Plasma total NEFA concentration was determined by the enzymatic method in 10, 9, and 9 dogs in the Omegaven, Intralipid and saline groups, respectively. One dog of the Intralipid group had a pre-treatment value that was far beyond the upper limit of the reference range of Wako analysis. In addition, the pre-treatment value was more than two standard deviations of the mean value of the corresponding GC result. The NEFA determinations from this dog in both the enzymatic and GC analysis were excluded from the evaluation. Data of another dog were excluded due to insufficient NEFA extraction for GC analysis in the 5 hours post-surgery sample. As a result, GC analysis sample size in each group was 10, 8, and 9 in Omegaven, Intralipid and saline groups, respectively.

By both the enzymatic and GC analyses, as shown in Figure 4, plasma total NEFA concentration across all treatment groups was elevated significantly (p<0.001) in 5 hours post-surgery plasma compared to pre-surgery or 24 hours post-surgery. No significant difference was seen between pre-surgery and 24 hours post-surgery plasma
total NEFA concentrations.

There were no significant treatment group differences among pre-surgery and 5 hours post-surgery plasma NEFA concentrations. Plasma NEFA concentrations in dogs of the saline group were higher than those of the Intralipid group at the 24 hours sampling time as indicated by the enzymatic (p=0.003) and GC (p=0.011) methods.

Correlation between the results of the enzymatic and GC analyses is illustrated in Figure 5. Pre-surgery and 24 hours post-surgery plasma NEFA observations had high correlations between the methods (r² = 0.76 p=0.000 in pre-surgery and r²=0.74 p=0.000 in 24 hours post-surgery). The 5 hours post-surgery plasma NEFA observations compared to the other sampling times had a lower but significant correlation between the methods (r²= 0.51 p=0.000).

Fatty acids profile in red blood cell membranes

Only AA and EPA concentrations were evaluated in erythrocytes. Concentrations of FA were expressed relative to the number of red blood cells isolated from pre-surgery blood samples. There was no significant difference of AA concentration among the groups (Figure 6). There was no significant difference of EPA concentration among the groups (Figure 6). Absolute recovery rate of both EPA and AA was 8.7%. The relative recovery rate of EPA against internal standard
(margaric acid, 17:0) was 79%. The relative recovery rate of AA against internal standard (lauric acid, 12:0) was 81%. In chromatograms, the peak of DHA was not completely separated from that of linoleic acid (18:2n-6); therefore, the concentration and recovery rate of DHA could not be determined.

**Non-esterified fatty acids profile in plasma**

The proportions and concentrations of non-esterified FA found in plasma of dogs in each treatment group at each sampling time are shown in Table 4a and 4b. The FA of principal interest in this study were AA, EPA, DHA and the total of long-chain omega-3 FA (total LC omega-3). Total LC omega-3 contains EPA, DHA and docosapentaenoic acid (DPA), which is an intermediate of EPA and DHA. Therefore, proportions of these FA were compared across all sampling times and among the infusion treatment groups as shown in Figure 7. Sample sizes were 10, 8 and 9 for Omegaven, Intralipid and saline groups, respectively.

**Observations on AA** - Across treatments, 5 hours post-surgery AA concentrations were higher than both pre-surgery (p=0.000) and 24 hours post-surgery (p=0.007) concentrations by a mean of approximately 30%. There was no significant difference between pre-surgery and 24 hours post-surgery plasma AA concentrations. There was no significant treatment effect on plasma AA at any sampling time.
**Observations on EPA** - Among dogs given Omegaven the 5 hours post-surgery EPA concentrations (8.8 ± 1.0 µM, mean ± standard error of the mean, [SEM]) were significantly and substantially higher than pre-surgery (p<0.001) and 24 hours post-surgery (p<0.001) concentrations, and they were higher than those of Intralipid (p<0.001) and saline groups (p<0.001) (0.7 ± 0.3 and 1.6 ± 0.8 µM in Intralipid and saline groups, respectively, mean ± SEM). Pre-surgery and 24 hours post-surgery plasma EPA concentrations were not significantly different for all treatment groups. The plasma EPA concentrations observed in dogs of the Intralipid and saline groups were not significantly different all sampling times.

**Observations on the ratio of 5 hour post-surgery AA to EPA** - The mean ratio of 5 hours post-surgery plasma concentration of AA to EPA in dogs of the Omegaven group was 2:1 and significantly lower than those in dogs of the Intralipid (p=0.002) and saline (p=0.014) groups, which were 9:1 and 6:1, respectively.

**Observations on DHA** - As seen with plasma EPA concentrations, the 5 hours post-surgery DHA concentrations in dogs of the Omegaven group (13.6 ± 1.8 µM, mean ± SEM) were significantly higher than the DHA concentration of the pre-surgery (p<0.001) and 24 hours post-surgery (p<0.001) concentrations. The 5 hours post-surgery DHA concentrations in dogs of the Omegaven group were also higher than those in dogs of the Intralipid group (2.3 ± 0.6 µM, mean ± SEM,
p<0.001) and saline group (2.6 ± 1.0 μM, mean ± SEM, p<0.001). The pre-surgery and 24 hours post-surgery DHA concentrations were not significantly different among the treatment groups. There was no significant difference in DHA concentrations across all sampling times for dogs in saline group. For dogs of the Intralipid group, DHA concentrations at the 5 hours post-surgery were significantly greater than 24 hours post-surgery (p=0.014), whereas there was no significant difference between pre-surgery and 5 hours post-surgery and pre-surgery and 24 hours post-surgery for plasma DHA concentrations.

**Observations on total long-chain omega-3 fatty acid (total LC omega-3)** -

The 5 hours post-surgery total LC omega-3 concentrations in dogs of the Omegaven group (25.1 ± 3.0 μM, mean ± SEM) were significantly higher than those concentrations for the pre-surgery and 24 hours post-surgery (p<0.001 in both pre-surgery and 24 hours post-surgery) sampling times. The 5 hours post-surgery total LC omega-3 concentrations in dogs of the Omegaven group were higher than those of the same sampling time for dogs in the Intralipid (4.2 ± 1.0 μM, p<0.001) and saline (5.9 ± 2.0 μM, p<0.001) groups. For dogs in the Intralipid group, the 5 hours post-surgery total LC omega-3 concentrations were also higher than those concentrations of the pre-surgery and 24 hours post-surgery (p=0.004 in pre-surgery, p=0.015 in 24 hours post-surgery) sampling times. Among dogs of within each group,
pre-surgery and 24 hours post-surgery total LC omega-3 concentration were not significantly different. For dogs of saline group, the total LC omega-3 concentrations at all sampling times were not significantly different.

**Leukocyte cytokine production capacity assay**

**IL-6 production from whole blood cultures** - The IL-6 productions stimulated with LPS and LTA were significantly greater than PBS for all treatment groups (p<0.002). At 5 hours post-surgery, IL-6 productions stimulated with all PAMPs (LPS, LTA and PG) were significantly greater than pre-surgery in saline group (p<0.001) as shown in Figure 8a. In the Intralipid group, IL-6 production stimulated with LPS at 5 hours post-surgery was less than that in Omegaven (p=0.048) and saline group (p=0.001), whereas there was no significant difference in IL-6 production between Omegaven and saline group. At both pre-surgery and 24 hours post-surgery, IL-6 concentration stimulated with LPS did not differ among the treatment group.

**IL-10 production from whole blood** - Whole blood IL-10 productions stimulated with LPS and LTA were significantly greater than PBS in all treatment group (p<0.05). As shown in Figure 8b, in saline group, IL-10 productions stimulated with LPS and LTA were significantly greater at 5 hours post-surgery compared to pre-surgery (p<0.013). The sampling-time differences in IL-10 production stimulated
with all PAMPs did not differ among the treatment groups.

**TNFα production whole blood** – The LPS and LTA stimulated significant production of TNFα compared with PBS for all treatment groups (p<0.02). As shown in Figure 8c, in saline group, LPS and PG-stimulated TNFα productions at 5 hours post-surgery did not differ from pre-surgery, and with LTA stimulation 5 hours post-surgery TNFα production was significantly less than pre-surgery (p<0.016). In the Omegaven group, TNFα productions stimulated with LTA and PG at 5 hours post-surgery were significantly less than pre-surgery (p<0.004). The sampling time difference in TNFα production stimulated with all PAMPs did not differ among all treatment groups.
Chapter 4
Discussion

As expected, plasma omega-3 FA (EPA, DHA and total LC omega-3) concentrations were significantly increased after fish oil emulsion (Omegaven) infusion at the 5 hours post-surgery time point. The lipid emulsion that was studied (Omegaven) consisted of lipid droplets of TG and egg phospholipids. Most of the FA contained in the lipid emulsion were in the form of TG. There were very small amounts of omega-3 and other PUFA in the phospholipids of the emulsion, but they are quantitatively negligible according to the manufacturer. Hydrolysis of TG of the infused fish oil emulsion may have freed DHA and EPA, causing the specific increases of these FA in the NEFA fraction of plasma. Activity of LPL in muscle vasculature, which was likely increased with food deprivation among the dogs, may have been most responsible for catalyzing hydrolysis of TG to free the EPA and DHA.

Mean concentrations of both non-esterified EPA and DHA after surgery increased by more than four times compared to their concentrations before surgery in dogs infused with Omegaven. In comparison to the Intralipid and saline infusions, mean non-esterified EPA and DHA concentrations after Omegaven infusions were increased by more than five times. The increases in non-esterified EPA and DHA observed after Omegaven infusion was marked but transient.
The mean ratio of non-esterified AA to non-esterified EPA in the Omegaven group 5 hours post-surgery was 2:1. This ratio differed significantly from the ratios of 9:1 and 6:1 in the Intralipid and saline groups, respectively. Given that EPA competes with AA in eicosanoid synthesis, the observed elevation in non-esterified EPA following fish oil emulsion infusion may be of physiological importance, because EPA-derived eicosanoids are generally believed to be less inflammatory compared to those derived from AA.65

In this study, the specific increases in EPA and DHA after fish oil emulsion infusion were seen promptly (5 hours post-surgery) and waned by 24 hours after the surgical trauma of OVH. This observation indicates that the effects of fish oil emulsion infusion might occur quickly, and not be of long-lasting duration. Therefore, infusion of fish oil emulsion might be beneficial to attenuate the systemic inflammatory response only soon after trauma. Given that non-esterified EPA and DHA are substantial substrates for production of less inflammatory lipid mediators, and also compete with AA in lipid mediator synthesis, acute administration of EPA and DHA by fish oil has a potential advantage over feeding a diet enriched in EPA and DHA in attenuating acute systemic inflammatory response soon after trauma.

As expected, total NEFA concentration was increased immediately after OVH in this study. An increase of approximately 30% was observed. Because of the
relatively minor surgical trauma of an OVH, the magnitude of the NEFA increase was less than that of sepsis and chronic enteritis in previous reports.\textsuperscript{66,67} In these previous reports, sepsis and chronic enteritis caused approximately 60% increase in plasma NEFA concentration. At 5 hours post-surgery, the magnitude of increase in NEFA concentration did not differ with type of infusate used. Hence, the fish oil infusion and the EPA and DHA it provided were not effective in reducing the post-OVH elevation in plasma NEFA. This result is not surprising given the mechanism believed to cause increases of NEFA after trauma. In addition, given that during the fasting period plasma NEFA concentration is increased compared to postprandial, fasting NEFA concentration (pre-surgery) in this study, nevertheless, was greater than previously reported.\textsuperscript{68,69} Therefore, the post-OVH plasma NEFA concentration may have been too high to observe the effects of the EPA and DHA provided by fish oil infusion.

The increases of total NEFA concentration after surgery was possibly due to increased hydrolysis of stored and lipoprotein TG in response to the surgical trauma. Surgery is known to induce lipolysis through stimulation of the sympathetic nervous system.\textsuperscript{34} Catecholamines released in response to surgical trauma increase β-adrenergic receptor activity on adipocyte membranes and thereby stimulate HSL activity, resulting in increased intra-adipocyte lipolysis.\textsuperscript{70} In a minor way, intravascular LPL activity may have also contributed to the post-surgical increase of
plasma total NEFA concentration in dogs infused with the lipid emulsion. Additionally, the surgical trauma may have activated PLA₂ resulting in hydrolysis of lipoproteins and release of FA into circulation. We used EDTA-containing tubes to separate plasma to avoid iatrogenic lipolysis. Heparin is known to activate LPL, which may be released into circulation, and cause intravascular lipolysis.⁷¹

The total plasma NEFA concentration was determined by two methods in this study. To my knowledge, this is the first study to compare total plasma NEFA concentration by two different methods. The mean total plasma NEFA concentrations determined with the enzymatic analysis were greater than those determined by GC in all treatment groups. One possible reason for the difference in method outcomes might be the difficulty of retrieving all plasma FA in GC analysis due to inevitable loss of FA during the fatty acid extraction procedure. During the plasma sample preparation procedure for GC analysis, NEFA were separated from lipid extract that also contained TG and phospholipids. We examined if NEFA were eluted in the same fraction of TG and phospholipids during the lipid separation procedure. We did not find any loss of NEFA in this step of the GC analysis. In contrast, during the enzymatic analysis, plasma was used directly for enzyme reactions so that a lipid extraction step was not needed as in GC analysis, and NEFA were not lost in the sample preparation process.
Another possible explanation for the higher mean values in enzymatic compared to GC analysis might be the following. The incubation procedure in enzymatic analysis may have resulted in some lipolysis mediated by PLA$_2$ activity in response to surgical trauma. In contrast, NEFA were extracted from plasma soon after thawing for the GC analysis so that additional lipolysis from plasma enzyme activity could not have occurred.

Lipolysis during the incubation step of the enzymatic analysis may also explain the uniquely low correlation of 5 hours post-surgery total plasma NEFA concentration between enzymatic and GC analyses. Surgical trauma may have resulted in production of acute phase proteins, such as secreted PLA$_2$ (sPLA$_2$). Activity of sPLA$_2$ may have released NEFA from plasma phospholipid-rich lipoprotein, such as high-density lipoprotein (HDL), during incubation of plasma used in the enzymatic analysis. The amount of sPLA$_2$ released may have varied among the dogs and reflected the severity of surgical trauma. Although the surgical trauma of OVH is relatively low, variation in trauma likely occurred. Those dogs with more trauma might have had more activity of sPLA$_2$, which caused more increase in post-surgical total NEFA concentration during the incubation step of the enzymatic analysis. The plasma sPLA$_2$ activity may have been normalized by 24 hours after surgical trauma, so that 24 hours post-surgery total NEFA concentration was less
influenced by the activity of sPLA2 during the incubation step of the enzymatic analysis.

Leukocyte cytokine production capacity was used as a surrogate marker of in vivo inflammatory state. After surgery, PAMPs-stimulated IL-6 and IL-10 productions were increased compared to before surgery. In contrast, PAMPs-stimulated TNFα productions did not change (LPS and PG) or were decreased (LTA). As such, that surgery resulted in increases of leukocyte production capacity of IL-6 and IL-10, but not TNFα. At 5 hours post-surgery, with LPS stimulation, blood cultures from dogs given the Intralipid emulsion had less IL-6 production than cultures from dogs given either the Omegaven emulsion or saline. This observation may indicate that LPS-induced IL-6 production by leukocytes in response to surgical trauma is not suppressed by fish oil infusion, or the soybean oil emulsion may have anti-inflammatory effect. Since we did not evaluate if infused omega-3 FA were incorporated into immune cell membrane at this time point (5 hours post-surgery), omega-3 FA may not have altered intracellular signaling pathway or transcriptional factor activation of immune cells to show the treatment effects during our the period. Given that each dog may have physiological variance and different sensitivity for the assay, the mean difference of LPS-stimulated IL-6 production between Omegaven and Intralipid groups at 5 hours post-surgery may not have clinical significance.
Dietary supplementation of omega-3 FA, but not omega-6 FA, downregulated experimentally induced ear inflammation by enhancing IL-10 production in lymphocytes in a murine model. On the other hand, a previous study with fish oil lipid emulsion reported that omega-3 FA administration decreased isolated macrophage IL-10 production exposed to LPS. In the present study, though surgery increased IL-10 production, we did not find a treatment effect. As seen in IL-6, fatty acid composition of immune cell membranes may not have been altered by fish oil infusion to show treatment effects, so other lipid anti-inflammatory lipid mediators may need to be evaluated.

Inverse association of total plasma PUFA concentration with TNFα was reported in a previous study. In our study, the Omegaven group had a significantly greater total PUFA concentration at 5 hours post-surgery than pre-surgery, and TNFα productions stimulated with LTA and PG at 5 hours post-surgery were significantly less than pre-surgery. This finding supports a previous report. However, the trend in changes of PAMPs-stimulated TNFα production from pre-surgery to 5 hours post-surgery did not differ among treatment groups. In a previous study, it was reported that IL-10 acts as a deactivating cytokine of macrophages that inhibits LPS-induced TNFα production by suppressing of TNFα gene expression. Given that TNFα production was not apparently increased after surgery, increased IL-10
production in response to surgery may have inhibited TNFα production by circulating leukocytes, rmasking fish oil effects. Since the OVH trauma was relatively minor, inflammation followed by surgical trauma was probably not great enough to exaggerate the fish oil effect.

Fatty acid concentration in erythrocytes reflects the history of dietary FA intake by the dogs over the lifespan of the erythrocytes (110 to 120 days). At admission, owners were questioned about the diet they usually gave to their dogs. Although many commercial dog foods may contain omega-3 FA, answers to survey questions indicated the diets of the study dogs were low in ingredients containing fish products and, therefore, also in EPA and DHA. The data on RBC membrane FA content in the study dogs did not indicate any significant difference in EPA concentration among the treatment groups. Hence, substantial group differences in endogenous background omega-3 FA concentration did not confound testing for an effect of fish oil.

The concentration of EPA and AA in RBCs appeared low in the present study compared to previous reports. A dietary requirement for long-chain omega-3 FA is established for dogs because of insufficient de novo synthesis of those FA from the shorter-chain vegetable source omega-3 fatty, α-linolenic acid. Nevertheless, the Association of American Feed Control Officials (AAFCO) presently does not
recommend dietary supplementation of EPA or DHA. Therefore, pet food companies are not compelled to include omega-3 FA in their diets and it is common for commercial dog foods to contain low concentrations of EPA and DHA.

The AA concentration in RBCs did not differ among treatment groups in the present study. This finding was a desired outcome because AA competes with EPA in formation of lipid mediators that affect inflammation. Group difference in AA availability might have confounded testing for an effect of fish oil.

With the HPLC method presently used, a clearly definable DHA peak was not detected. This is because of close elution of the fluorescent derivative of DHA with that of linoleic acid (18:2n-6) precluding quantitation. Though not quantifiable, DHA concentration in RBCs was assumed to be low in the dogs and similar among the treatment groups. According to a previous study in dogs, the proportion of DHA among FA in RBC membranes is approximately 0.25 to 0.3 mol% of the total of RBC membrane FA. Given this previous observation and the low concentration of DHA typically in commercial dog foods, quantitation of erythrocyte DHA may not have been possible with the HPLC method used even if clear peak separation was achievable.

Non-esterified AA concentration was greater in 5 hours post-surgery plasma than pre-surgery and 24 hours post-surgery plasma in all treatment groups in this
study. The higher concentration of non-esterified AA in 5 hours post-surgery plasma compared to pre-surgery plasma could be explained by three conditions: (1) In the case of fish oil infusions, 4% of .the FA in the product used (Omegaven) was AA. Some of the non-esterified AA may have come from the fish oil emulsion. (2) Some of the non-esterified AA may have been cleaved from endogenous AA in lipoprotein phospholipids in plasma. (3) Some of the non-esterified AA may have been from macrophage and other cellular mobilization of membrane AA following surgical trauma.78

In a previous study,79 plasma free AA concentration was decreased and the plasma free omega-3/omega-6 ratio was reversed toward favoring of EPA and DHA over AA within two days of fish oil emulsion infusion during a 5-day infusion protocol. However, in our study AA concentrations in plasma at 5 and 24 hours post-surgery were not significantly lower in the fish oil emulsion group compared to other treatment groups. Our study differed possibly due to a shorter period of infusion (3 hours) compared to the previous study or a difference of infusion protocol. In the study of Mayer et al 79 septic patients were given a fish oil emulsion (Omegaven) at a dose of 400 ml/day, divided into three periods so that plasma omega-3 FA concentration could be maintained throughout a 5-day infusion period.

Our study had limitations centered around sample size. Sample size was
smaller than we planned at the beginning of the study due to relatively strict inclusion
criteria. In addition, two dogs were excluded from this study due to the use of
ephedrine during surgery. Vasopressor agents, such as ephedrine, can mediate
lipolysis and cause fatty acid release from adipose tissue and lead to iatrogenic
elevation of plasma NEFA concentration after surgery. Therefore, low statistical
power of the study may have prevented discovery of treatment effects, especially for
observations on cytokines when between-individual variation was large.

The FA composition of the presently used lipid emulsions should have been
determined. Each lipid emulsion contains a natural oil, so there is expectedly a
variation in FA content between manufacturing lots. Therefore, the FA compositions
of the lots of fish oil and soybean emulsions used in this study should have been
determined for future reference.

Analgesic agents, such as opioids and α2-adrenergic agonists (i.e.,
dexmedetomidine) have been reported to modulate immune responses;80-85 however,
these agents were necessary for peri- and postoperative pain control. These agents
may have affected leukocyte cytokine production stimulated with PAMPs.

Because reproductive hormones may affect immune function as reported in a
previous study,86 we excluded dogs that were currently in estrus. However, serum
progesterone concentration was not determined. Strict determination of the stage of
the estrus cycle would have required serum progesterone assay. The variation of reproductive hormone concentration might have affected leukocyte cytokine production capacity induced by PAMPs.

Only leukocyte cytokine production capacity by cultured blood was utilized to evaluate inflammatory response in this study. Use of more direct markers of the systemic inflammatory state, such as acute phase proteins and lipid mediators (e.g., LT or resolvin), may have been better for evaluating effectiveness of the fish oil treatment.

The age of dogs was not significantly different among the treatment groups. However, because we did not set an upper limit for age, the range of age was wide compared to the common age of dogs having elective OVH. An elevation of TNFα production capability by cultured blood cells was observed in middle-aged dogs. However, according to Deitschel et al who reported on age effects on cytokine release in whole blood culture, our mean age in all treatment group would be categorized as young dogs.

**Summary**

Total NEFA concentration increased after surgical trauma, which agrees with previous reports. Increases of total NEFA concentration after surgical trauma is consistent with lipolysis being induced in response to surgical trauma. Plasma
non-esterified omega-3 FA concentrations markedly increased after fish oil emulsion infusion as reported in other studies. The mechanism underlying release of the omega-3 FA from the emulsion was not apparent. Cytokines released by PAMPs-stimulated cultured blood cells after surgery were not attenuated by fish oil infusion.

The OVH surgery affected the amount of cytokines released by PAMPs-stimulated cultured blood cells. This result indicated that OVH surgery has a systemic effect that can influence cytokine release by circulating leukocytes. The studied fish oil treatment and resultant transient changes in plasma non-esterified EPA and DHA concentration were mostly ineffective in altering cytokine production by cultured blood cells.

The present study indicates that infusion of fish oil emulsion at an effective dosage and duration used in human patients was not harmful to apparently healthy, young dogs undergoing an elective abdominal surgery. However, the fish oil infusion was not demonstratively beneficial for attenuating a systemic inflammatory response to the surgery, in as much as the production of cytokines by cultured blood cells indicated the systemic inflammatory response of the dogs to the trauma.
Docosahexaenoic acid is distributed mainly to brain and retina and is important to the growing fetus and infant. A previous study reported that supplementation with the fatty acid of interest may increase a specific omega-3 fatty acid concentration in plasma.\textsuperscript{90} Therefore, DHA-rich lipid emulsion could be used in acute brain trauma or pregnant dogs. In addition, different effects on immune function with DHA, EPA and $\alpha$-linolenic acid were reported in other studies.\textsuperscript{91,92} Further study of different formulations of fish oil emulsion is warranted. The relationship between low blood concentration of PUFA and inherited retinal degenerations was reported in Swedish briard dogs.\textsuperscript{93} Although we did not have this breed in our study, fatty acids composition may differ among breeds as reported in boxers and Doberman pinschers.\textsuperscript{94} Further study of the effects on fish oil emulsion infusion in breeds predisposed to certain diseases is warranted.

In this study, the same dosage used in human (0.2 g/kg BW) was used for dogs, and no predictable side effects were seen. However, there has been no report of appropriate parenteral dosage of fish oil emulsion in dogs for management of inflammation; therefore, further evaluation of dosage is needed. Although fish oil infusion did not change PAMPs-stimulated leukocyte cytokine production capacity in
the present study, other inflammatory mediators, such as lipid mediators and C-reactive protein, warrant evaluation.
Table 1: Composition of the lipid emulsions. Values represent ranges reported by the manufacturer.

<table>
<thead>
<tr>
<th>Lipid composition</th>
<th>Omegaven (fish oil)</th>
<th>Intralipid (soybean oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid composition</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Palmitic acid, 16:0</td>
<td>2.5-10</td>
<td>7.0-14.0</td>
</tr>
<tr>
<td>Stearic acid, 18:0</td>
<td>0.5-2</td>
<td>1.4-5.5</td>
</tr>
<tr>
<td>Oleic acid, 18:1n-9</td>
<td>6.0-13.0</td>
<td>19.0-30.0</td>
</tr>
<tr>
<td>Linoleic acid 18:2n-6</td>
<td>1.0-7.0</td>
<td>44.0-62.0</td>
</tr>
<tr>
<td>Linolenic acid 18:3n-3</td>
<td>&lt;2.0</td>
<td>4.0-11.0</td>
</tr>
<tr>
<td>Arachidonic acid 20:4n-6</td>
<td>1.0-4.0</td>
<td>0</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (EPA) 20:5n-3</td>
<td>12.5-28.2</td>
<td>0</td>
</tr>
<tr>
<td>Docosahexaenoic acid (DHA) 22:6n-3</td>
<td>14.4-30.9</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2: Signalments of dogs in each treatment group. Age, body weight, body condition score (BCS, 1-9) and breeds of dogs were compared among treatment groups. Data are expressed as the medians and ranges, where ranges and number of dogs are presented in parentheses. There is no significant difference of age (p=0.755), body weight (p=0.242), and BCS (p=0.875) among the treatment groups. [BCS: body condition score is the assessment whether a dog is the correct weight or not, and based on a scale running from 1-9, e.g. 1 = emaciated, 9 = grossly obese]

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Omegaven</th>
<th>Intralipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>6.0 (4-34, n=9)</td>
<td>9.0 (4-61, n=10)</td>
<td>9.5 (5-35, n=10)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>10 (5.2-13.4, n=9)</td>
<td>14 (8.1-17.7, n=10)</td>
<td>12 (4.3-20.4, n=10)</td>
</tr>
<tr>
<td>BCS</td>
<td>5.0 (4-6, n=8)</td>
<td>5.0 (3-6, n=10)</td>
<td>5.0 (4-6, n=10)</td>
</tr>
<tr>
<td>Breeds (n=10 for Omegaven and Intralipid, n=9 for saline group)</td>
<td>Boston terrier, Irish terrier, Mix, Boykin spaniel, Australian shepherd, Shih tzu</td>
<td>Mix, PUG, Gordon shorthaired pointer, Jack russel terrier, American staffordshire terrier</td>
<td>Boston terrier, Mix, Australian cattle dog, Bichon frise, Alaskan malamute</td>
</tr>
</tbody>
</table>
Table 3: Experimental design and blood collection schedule. Blood samples are collected at three different times, pre-surgery (pre), 5 hours post-surgery (5h post-surgery) and 24 hours post-surgery (24h post-surgery). Pre-surgery samples were collected after 12 hours of food withholding.

<table>
<thead>
<tr>
<th>Day1 (Admission)</th>
<th>Day2 (Surgery + infusion)</th>
<th>Day3 (Dismissal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12hrs fasting before surgery</td>
<td>Surgery</td>
<td>Infusion for 3 hrs</td>
</tr>
<tr>
<td>Blood collecting (pre)</td>
<td>Blood collecting after 30min washout (5h post-surgery)</td>
<td>Blood collecting 24hrs after Surgery (24h post-surgery)</td>
</tr>
</tbody>
</table>
Table 4a: Plasma non-esterified fatty acids composition of treatment groups. Data are expressed as percentage of total FA, mean ± SEM (n=10, 8, 9 in Omegaven, Intralipid and saline groups, respectively). Values with different superscript letters in a row are significantly different at p<0.017 by Bonferroni correction. Lower case letters designate the comparisons between time points within each treatment group. Upper case letters show within treatment group comparisons at each time point. [FA: fatty acids, SEM: standard error of the mean, Pre: pre-surgery, 5h post-surgery: 5 hours post-surgery, 24h post-surgery: 24 hours post-surgery]

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre</th>
<th>5h post-surgery</th>
<th>24h post-surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Saline</td>
<td>Intralipid</td>
</tr>
<tr>
<td>14:0</td>
<td>2.0±0.2</td>
<td>1.9±0.2</td>
<td>2.5±0.4</td>
</tr>
<tr>
<td>16:0</td>
<td>28.4±1.6</td>
<td>29.5±1.7</td>
<td>28.8±2.2</td>
</tr>
<tr>
<td>18:0</td>
<td>13.5±1.9</td>
<td>14.9±1.7</td>
<td>14.9±2.0</td>
</tr>
<tr>
<td>16:1</td>
<td>4.1±0.5</td>
<td>4.4±0.7</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td>18:1n9</td>
<td>31.3±1.8</td>
<td>30.2±2.1</td>
<td>30.2±2.2</td>
</tr>
<tr>
<td>18:2n6</td>
<td>16.3±1.5</td>
<td>14.9±1.3</td>
<td>15.3±1.8</td>
</tr>
<tr>
<td>18:3n6</td>
<td>0.1±0.02</td>
<td>0.1±0.02</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>18:3n3</td>
<td>1.5±0.4</td>
<td>0.6±0.1</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>20:2n6</td>
<td>0.6±0.3</td>
<td>0.2±0.2</td>
<td>0.4±0.2</td>
</tr>
<tr>
<td>20:3n6</td>
<td>0.1±0.04</td>
<td>0.1±0.03</td>
<td>0.2±0.02</td>
</tr>
<tr>
<td>20:4n6</td>
<td>1.3±0.3</td>
<td>2.1±0.6</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>20:5n3</td>
<td>0.2±0.1</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>22:4n6</td>
<td>0.3±0.2</td>
<td>0.5±0.3</td>
<td>0.1±0.03</td>
</tr>
<tr>
<td>22:5n3</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>22:6n3</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.5±0.2</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>20.7±1.6</td>
<td>19.1±1.3</td>
<td>19.8±2.0</td>
</tr>
<tr>
<td>n-6 FA/n-3 FA</td>
<td>16.7±4.2</td>
<td>17.4±5.0</td>
<td>10.3±1.6</td>
</tr>
<tr>
<td>Total SAT FA</td>
<td>43.9±3.5</td>
<td>46.3±3.2</td>
<td>46.2±4.3</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>35.3±2.3</td>
<td>34.7±2.7</td>
<td>34.0±2.6</td>
</tr>
</tbody>
</table>
Table 4b: Plasma non-esterified fatty acids expressed in concentrations units (µM).

Data expressed as concentration (µM) of FA and mean ± SEM (n=10, 8, 9 in Omegaven, Intralipid and saline groups respectively). Values with different superscript letters in a row are significantly different at p<0.017 by Bonferroni correction. Lower case letters designated the comparisons between time points. Upper case letters show the within treatment group comparisons at each time point. [FA: fatty acids, SEM: standard error of the mean, Pre: pre-surgery, 5h post-surgery: 5 hours post-surgery, 24h post-surgery: 24 hours post-surgery]
Figure 1: Chemical structures of omega-3 and omega-6 fatty acids.

Omega-3 fatty acid, eicosapentaenoic acid (EPA, 20:5n-3)


Omega-6 fatty acid, linoleic acid (18:2n-6)

Figure 2: Metabolism of omega-6 and omega-3 fatty acids. Omega-6 (linoleic acid, LA) and omega-3 fatty acids (α-linolenic acid, ALA) from dietary sources are converted to the longer chain, more unsaturated FA, such as arachidonic (AA) and eicosapentaenoic acid (EPA). The AA and EPA are substrates for synthesis of eicosanoids (prostaglandins [PG], thromboxanes [TXA], leukotrienes [LT]) and are metabolized by COX and LOX to produce the different type of eicosanoids. The eicosanoids derived from AA (2-series of PG and TXA and 4-series of LT) are more pro-inflammatory and that those from EPA (3-series of PG and TXA, and 5-series of LT). The conversion to DHA is processed by peroxisomal β-oxidation reaction. [FA: fatty acids, COX: cyclooxygenase, LOX: 5-lipoxygenase]

Figure 3: Omega-3 fatty acids inhibit the nuclear factor –kappa B signaling pathway. There are three stages of inhibition. (1) Omega-3 FA interfere with TLR 4 and 2 by LPS. (2) Omegaven inhibits IKK phosphorylation which prevents activation of the NF-kB pathway. (3) Omega-3 FA act as an agonist of PPAR, which interfere with NF-kB DNA binding activity. [NF-kB: nuclear factor- kappa B, TLR: toll-like receptor, LPS: lipopolysaccaride, TRAF: TNF-associated factor, IkB: I Kappa B, IKK: I Kappa B kinase, PPAR: peroxisome proliferator-activated receptor, TNF: tumor necrosis factor-alpha]
Figure 4: Comparison of total non-esterified fatty acids concentration in infusion groups by enzymatic (Wako kit, the solid bar graph) and gas chromatography (GC, the pattern bar graph) analyses. At 5 hours post-surgery (5h post-surgery), total NEFA was significantly higher than that at pre and 24 hours post-surgery (24h post-surgery) by both of analyses (p<0.001). Each box in the figure represents the mean value and the bar in each box represents SEM. The table below represents the mean and SEM value of each group in two methods. [NEFA: non-esterified fatty acids, SEM: standard error of the mean, pre: pre-surgery, 5h post-surgery: 5 hours post-surgery, 24h post-surgery: 24 hours post-surgery]

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Omegaven</th>
<th>Intralipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymatic method (Wako)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>948.6 ± 103.7</td>
<td>845.5 ± 129.4</td>
<td>698.7 ± 125.4</td>
</tr>
<tr>
<td>5h post-surgery</td>
<td>1531.2 ± 112.4</td>
<td>1459.4 ± 172.6</td>
<td>1351.5 ± 165.9</td>
</tr>
<tr>
<td>24h post-surgery</td>
<td>1008.0 ± 81.8</td>
<td>793.3 ± 141.9</td>
<td>703.8 ± 64.8</td>
</tr>
<tr>
<td><strong>Gaschromatography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>788.5 ± 127.9</td>
<td>720.8 ± 125.6</td>
<td>579.0 ± 107.5</td>
</tr>
<tr>
<td>5h post-surgery</td>
<td>1215.4 ± 115.9</td>
<td>1076.8 ± 116.0</td>
<td>1047.7 ± 118.7</td>
</tr>
<tr>
<td>24h post-surgery</td>
<td>836.4 ± 83.6</td>
<td>615.2 ± 86.0</td>
<td>595.1 ± 34.6</td>
</tr>
</tbody>
</table>

The mean ± SEM of total NEFA concentration (µM) in two methods.
Figure 5: Correlation of total non-esterified fatty acids concentration between the enzymatic (Wako) and gas chromatography (GC) analyses. All correlation values were significant (p<0.05). The correlation of 5h post was poor (r²=0.51) between Wako and GC. [Pre: pre-surgery, 5h post: 5 hours post-surgery, 24h post: 24 hours post-surgery]
Figure 6: Fatty acid concentrations in erythrocytes. Each box represents the mean value and the bar in each box represents SEM. The table below represents the mean and SEM values of arachidonic acid (AA) and eicosapentaenoic acid (EPA) in each group. There was no significant difference in neither AA (p=0.970) nor EPA (p=0.542) concentration among treatment groups. [SEM: standard error of the mean]

![AA concentration in RBC](image1)

![EPA concentration in RBC](image2)

The mean ± SEM of AA and EPA concentration (µmol/10⁶ RBC) in all groups.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Omegaven</th>
<th>Intralipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>12.6 ± 4.1</td>
<td>12.5 ± 3.9</td>
<td>15.2 ± 5.7</td>
</tr>
<tr>
<td>EPA</td>
<td>3.4 ± 0.8</td>
<td>6.4 ± 1.8</td>
<td>4.9 ± 1.2</td>
</tr>
</tbody>
</table>
Figure 7: Arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and total long-chain omega-3 fatty acid (total LC omega-3) concentration in plasma. Each box represents the mean value and the bar in each box represents SEM. The tables below represent the mean and SEM values of plasma AA, EPA, DHA and total LC omega-3 concentrations in all groups. Five hour post of AA concentration was significantly greater than pre (p=0.000) and 24h post (p=0.007). However, 5h post AA concentration in Omegaven did not differ from that in Intralipid (p=0.764) and saline (p=0.624). In contrast, 5h post EPA, DHA, and total LC omega-3 concentrations in Omegaven were significantly greater than that in Intralipid and saline (p=0.000). And also, in Omegaven, 5h post EPA, DHA, and total LC omega-3 concentrations were significantly greater than those of pre and 24h post (p=0.000). The ratio of 5h post AA to EPA in Omegaven (2:1) was significantly different from that in Intralipid (9:1, p=0.002) and saline (6:1, p=0.014). [SEM: standard error of the mean, pre: pre-surgery, 5h post: 5 hours post-surgery, 24h post: 24 hours post-surgery]

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Omegaven</th>
<th>Intralipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre</td>
<td>10.0 ± 1.9</td>
<td>11.1 ± 2.2</td>
<td>11.0 ± 3.7</td>
</tr>
<tr>
<td>5h post</td>
<td>14.2 ± 2.3</td>
<td>15.5 ± 2.7</td>
<td>14.0 ± 1.9</td>
</tr>
<tr>
<td>24h post</td>
<td>13.4 ± 2.4</td>
<td>10.2 ± 1.7</td>
<td>12.2 ± 2.6</td>
</tr>
</tbody>
</table>

The mean ± SEM of plasma AA concentrations (µM) in all groups.
The mean ± SEM of plasma EPA concentrations (µM) in all groups.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Saline (µM)</th>
<th>Omegaven (µM)</th>
<th>Intralipid (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre</td>
<td>1.0 ± 0.5</td>
<td>1.5 ± 0.4</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>5h post</td>
<td>1.6 ± 0.8</td>
<td>8.8 ± 1.0</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>24h post</td>
<td>1.5 ± 0.9</td>
<td>1.2 ± 0.5</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>
The mean ± SEM of plasma DHA and total LC omega-3 concentrations (µM) in all groups.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Omegaven</th>
<th>Intralipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DHA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>2.0 ± 0.7</td>
<td>2.9 ± 0.8</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>5h post</td>
<td>2.6 ± 1.0</td>
<td>13.6 ± 1.8</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>24h post</td>
<td>2.5 ± 1.1</td>
<td>2.7 ± 1.0</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td><strong>Total LC omega-3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>3.8 ± 1.5</td>
<td>5.5 ± 1.4</td>
<td>3.1 ± 1.5</td>
</tr>
<tr>
<td>5h post</td>
<td>5.9 ± 2.0</td>
<td>25.1 ± 3.0</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>24h post</td>
<td>5.2 ± 2.3</td>
<td>5.0 ± 1.6</td>
<td>2.5 ± 0.9</td>
</tr>
</tbody>
</table>
Figure 8a: Comparison of interleukin-6 production from whole blood culture after stimulation with PBS (control), LPS, LTA and PG in the three treatment groups. Each point represents the mean value and the bar in each point represents SD. At 5 hours post-surgery, IL-6 productions stimulated with all PAMPs (LPS, LTA and PG) were significantly greater than pre-surgery in saline group (p<0.001). In Intralipid group (square, dot line, ■), IL-6 production stimulated with LPS was significantly less than that in Omegaven (p=0.048) and saline (p=0.001) at 5 hours post-surgery. The asterisk below represents the significant difference in Intralipid compared to Omegaven and saline groups. Omegaven and saline groups were represented as diamond and solid line ( ■), and triangle and broken line ( ▲), respectively. [IL-6: interleukin-6, PBS: phosphate buffered saline, LPS: lipopolysaccharide, LTA: lipoteichoic acid, PG: peptidoglycan, SD: standard deviation, pre: pre-surgery, 5h post: 5 hours post-surgery, 24h post: 24 hours post-surgery]
Figure 8b: Comparison of interleukin-10 production from whole blood culture after stimulation with PBS (control), LPS, LTA and PG in the three treatment groups. Each point represents the mean value and the bar in each point represents SD. In saline group, IL-10 productions stimulated with LPS and LTA were significantly greater at 5 hours post-surgery compared to pre-surgery (p<0.013). The sampling time differences in IL-10 concentrations stimulated with LPS, LTA and PG did not differ among the treatment groups. Omegaven group was represented as diamond and solid line ( ). Intralipid group was represented as square and dot line ( ). Saline group was represented as triangle and broken line ( ). [IL-10: interleukin-10, PBS: phosphate buffered saline, LPS: lipopolysaccharide, LTA: lipoteichoic acid, PG: peptidoglycan, SD: standard deviation, pre: pre-surgery, 5h post: 5 hours post-surgery, 24h post: 24 hours post-surgery]
Figure 8c: Comparison of tumor necrosis factor-alpha production from whole blood culture after stimulation with PBS (control), LPS, LTA and PG in the three treatment groups. Each point represents the mean value and the bar in each point represents SD. In saline group, LPS and PG-stimulated TNFα productions at 5 hours post-surgery did not differ from pre-surgery, or with LTA stimulation it was significantly less than pre-surgery (p<0.016). In Omegaven group, TNFα productions stimulated with LTA and PG at 5 hours post-surgery was significantly less than pre-surgery (p<0.004). The sampling time differences in TNFα productions stimulated with LPS, LTA and PG did not differ among all treatment groups. Omegaven group was represented as diamond and solid line ( ). Intralipid group was represented as square and dot line ( ). Saline group was represented as triangle and broken line ( ). [TNFα: tumor necrosis factor-alpha, PBS: phosphate buffered saline, LPS: lipopolysaccharide, LTA: lipoteichoic acid, PG: peptidoglycan, SD: standard deviation, pre: pre-surgery, 5h post: 5 hours post-surgery, 24h post: 24 hours post-surgery]
REFERENCES


75. Armstrong L, Jordan N, Millar A. Interleukin-10 (IL-10) regulation of tumor necrosis factor alpha (TNF-alpha) from human alveolar macrophages and peripheral blood monocytes. Thorax 1996;51:143-149.


82. Venn RM, Bryant A, Hall GM, et al. Effects of dexmedetomidine on adrenocortical function, and the cardiovascular, endocrine and inflammatory resp

71


92. Anderson BM, Ma DW. Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis 2009;8:33.
