THE EFFECTS OF TEMPERATURE AND ADMIXTURE HANDLING ON LIPID EMULSION STABILITY IN CENTRALLY ADMINISTERED VETERINARY PARENTERAL NUTRITION (PN) ADMIXTURES

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by

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

LIST OF FIGURES v

LIST OF TABLES vi

ABSTRACT vii

Chapter

1. VETERINARY PARENTERAL NUTRITION: AN INTRODUCTION 1

    Part 1: Historical Focus……....1

    Part 2: Genesis of the Research……6

    Part 3: From Here to There….....11

2. A PRIMER ON PARENTERAL NUTRITION PART I: USES, INDICATIONS, AND COMPOUNDING 12

3. A PRIMER ON PARENTERAL NUTRITION PART II: FORMULATION, MONITORING, AND COMPLICATIONS 29

4. EFFECTS OF TEMPERATURE AND HANDLING CONDITIONS ON LIPID EMULSION STABILITY IN CENTRALLY ADMINISTERED VETERINARY PARENTERAL NUTRITION ADMIXTURES……55

5. FUTURE DIRECTIONS……71

APPENDIX

1. EXPANDED STATISTICAL DATA ANALYSIS 74

2. EXPANDED METHODS FOR EVALUATION OF LIPID PARTICLES USING ADOBE PHOTOSHOP WITH THE FOVEA PRO APPLICATION……...78

3. EXPANDED DESCRIPTION OF RADIUS AND ROUNDNESS DETERMINATION FOR EACH LIPID PARTICLE……...80

4. EXPANDED DISCUSSION OF STUDY LIMITATIONS……...82

5. TABLES 84

6. FIGURES 97

FOOTNOTES 100

REFERENCES CITED.....101

LIST OF FIGURES

Figure Page

1. Strict blood glucose monitoring protocol…….97

2. Mean ± SD percentage distribution of lipid particle diameters (determined via TEM examination at 0, 24, 48, 72, and 96 hours after preparation) in bags of PN admixture that were kept static at room temperature (approx 23^{o}C; control group) for 96 hours……..98

3. Oil layer in Group 2 venoset at 72 hours (continuously agitated group). Arrow indicates the oil layer……...99

4. Oil Layer in Group 2 intravenous pump cartridge at 72 hours (continuously agitated group). Arrow indicates the oil layer……...99

LIST OF TABLES

Table Page

1. A comparison of veterinary total parenteral and partial parenteral nutrition.....84
2. Indications for PPN……..84
3. Indications for TPN……..85
4. Guidelines for the initiation of nutritional support……..85
5. Technical guidelines for the administration of parenteral nutrition…….86
6. Sample calculation of TPN for a 10 kg dog that is suffering from pancreatitis…..87
7. Sample calculation of PPN for a 10 kg dog with a gastrointestinal foreign body that is going to surgery the next day for foreign body removal and enteral feeding tube placement…….89
8. A review of safety tips for administering parenteral nutrition PN)…….....91
9. Recommended potassium supplementation for hypokalemic patients……91
10. Blank TPN worksheet……..92
11. Blank PPN worksheet……..94
12. Mean ± SD and median (range) values of lipid particle diameter (determined via TEM examination at 0, 24, 48, 72, and 96 hours after preparation) in bags of PN admixture exposed to various conditions of temperature and handling (5 groups of 3 bags each)……95

EFFECTS OF TEMPERATURE AND HANDLING CONDITIONS ON LIPID EMULSION STABILITY IN CENTRALLY ADMINISTERED VETERINARY PARENTERAL NUTRITION ADMIXTURES

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ABSTRACT

Objective: To determine the temporal change in lipid particle size in veterinary parenteral nutrition (PN) admixtures kept at room temperature (23°C) versus admixtures filtered, refrigerated, and agitated.

Procedure: Fifteen 2 L bags of PN admixture containing 50% dextrose (525 mL), 20% lipid emulsion (453 mL), 8.5% amino acids/electrolyte solution (840 mL) and vitamin B complex (5 mL) were delivered through an intravenous pump (16 mL/hr) for 96 hours. Group 1 (n=3) was static, Group 2 (n=3) was continuously agitated, Group 3 (n=3) was agitated for 5 minutes every 4 hours, Group 4 (n=3) was static at 4°C, and Group 5 (n=3) was filtered (5 μm pore). After 96 hours, two 10 mL samples of PN (n=3) were cultured (bacterial). Samples (1.0 mL) were collected at 0, 24, 48, 72, and 96 hours and examined with transmission electron microscopy. Computer software (Adobe Photoshop & Fovea Pro) provided lipid particle diameters. Significance of time effects on size distribution was evaluated with Repeated Measures ANOVA.

Results: There was no significant difference in lipid particulate size among or within groups over time ($p \leq 0.05$). Group 2 separated into a visible oil layer by 72 hours. There was no bacterial growth (aerobic or anaerobic).

Conclusion: Lipid particulate size is stable in this veterinary PN admixture for more than 48 hours at 23°C. Manipulations of PN are unnecessary to prolong lipid particle stability; continuous agitation may hasten lipid breakdown.

Chapter I. **Veterinary parenteral nutrition: an introduction**

Part 1. Historical focus

> *All animals begin their lives in utero, receiving nourishment entirely by vein….*
>
> Stanley Dudrick, MD[1]

> *…[parenteral nutrition is] the intravenous administration of sufficient nutrients above the basal requirements to achieve tissue synthesis, positive nitrogen balance, and anabolism.*
>
> Flack JL, et al.[2]

The above two statements embody the conundrum that is parenteral nutrition (PN). On one hand the goal is very clear: to provide nutrients to a patient and allow the patient's metabolism to shift from a catabolic state to one where healing and tissue regrowth is possible. However, as with so many things in life, trying to simulate the human body and the intravenous feeding provided from mother to child is a difficult, if not impossible, goal to attain. Physicians working in pursuit of this goal have developed a complex field in human medicine that is constantly re-defining and changing the way nutrition is administered to patients.

The very first experiments with parenteral nutrition date from the 1600s when physicians used sharpened quills to administer milk and wine into the veins of dogs.[3,4] As expected, such experimentation did not yield favorable outcomes and the concept of intravenous nutrition was placed on hold for many years. Then, in the 1800s when cholera became a devastating cause of death amongst humans, a major breakthrough caused intravenous therapy to re-enter the medical consciousness. That breakthrough was the life-saving and groundbreaking use of intravenous saline solutions which made physicians acutely aware that administering medications intravenously was a viable treatment option.[4]

The next hurdle in the development of PN was to obtain the nutritional components in a form that could be utilized intravenously. The three basic building blocks of modern-day PN are dextrose and lipid sources to provide for the patient's caloric needs and amino acids to supply the body with anabolic compounds for protein synthesis. To this end, the first innovation came in the form of intravenous dextrose solutions.[4] The first experimentation with intravenous dextrose solutions was in 1915; this early work was expanded in the 1930s and 1940s to include both continuous infusions of dextrose and the administration of dextrose through the superior vena cava rather than a peripheral vein. The successful use of a central vein allowed researchers to deliver highly concentrated dextrose solutions (greater than 10%); previously, such hypertonic solutions could not be given through peripheral veins without causing damage to the vascular endothelium.[4]

On the heels of the creation of intravenous dextrose solutions came the development of protein hydrolysates in the 1930s and 1940s which provided protein in a form that could be administered intravenously.[3] These protein infusates became modified into our modern amino acid solutions in the 1970s.[4] All that was left then was development of another concentrated calorie source that could be utilized with dextrose to provide for the patient's caloric requirements. Several compounds were unsuccessfully tried including alcohol-containing solutions and various lipid solutions.[3,4] Finally in the 1960s, soybean-containing lipid emulsions were developed which fulfilled the need for another calorie source, breaking down the final barrier in the creation of the building blocks of modern parenteral nutrition.[3,4]

From these beginnings, it seemed that the development of total parenteral nutrition (TPN) designed to provide for all calorie needs of a patient could not be far away. However, in the 1960s, there were still several key hindrances to creating and using PN in human patients.[1] The first was the medical community's dogmatic conviction that nutrition had to be administered through a peripheral vein.[2] Phlebitis and venous thrombosis were common sequelae to the peripheral administration of intravenous solutions and patients uniformly found the process uncomfortable. Additionally, the usage of peripheral veins limited the concentration of dextrose that could be administered. A second large hurdle was the fact that the patient could not be administered more than 2500 to 3500 mL of liquid volume per day.[1] This severely restricted the volume of calories and protein that could be given to a patient and seemed to make it impossible to attain full caloric supplementation.

The remaining seemingly insurmountable impediments were all centered around the fact that the creation of protein, lipid, and dextrose sources to use intravenously were in their infancy and not as widely available or available in as many forms as needed.[1] These obstacles frustrated early researchers and physicians until the 1980s. At that point, technology had caught up with the dream of researchers and modern day lipid emulsions, dextrose solutions, and amino acid solutions were finally available and successfully combined into nutritional admixtures.

Fittingly, the first actual experimentation with TPN solutions was performed in beagles by Dr. Stanley Dudrick and his team over the course of several experiments in the 1960s. The dogs were given single solutions of TPN with all required amino acids, dextrose, electrolytes, lipids, vitamins, and minerals combined into a single solution.[3,4,5]

In so doing, much trial and error was required to both ensure that the various electrolytes did not precipitate with each other (especially calcium and phosphorus) and that the admixture was mixed aseptically. Additionally, this landmark work required the development of central venous catheterization in the dogs in order to administer the nutritional admixtures.[1] That the researchers were able to overcome every obstacle in their path was demonstrated by the results of their work: puppies fed parenterally for 72 to 256 days outweighed their orally-fed littermates and matched their littermates in skeletal growth, development, and activity levels.[6]

From here, the jump from dogs to humans came much faster than expected. In a bold move after seeing these positive findings in the beagles, Dudrick and his peers almost immediately began to use a modified version of the puppy feeding formulation in humans in 1966.[3,7] Six severely malnourished humans with diseases ranging from regional enteritis to bowel obstructions and pancreatitis were successfully fed for six days. During that time, the viability of TPN as a treatment modality seemed clear; all six patients displayed positive nitrogen balance, weight gain, normal wound healing, and increased strength and physical and mental activity levels, leading to their successful discharge from the hospital. In 1967, TPN was used for the first time in a human infant who was born with near total small bowel atresia leading to a duodenal-ileal anastomosis. The baby was fed successfully for a total of 22 months using TPN before her eventual death.[1,8]

Ironically, from these ostentatious beginnings in human medicine where experimentation on beagles played such a key role in the development of parenteral

nutrition, veterinary medicine did not seek to embrace PN until a publication in 1977.[9] In that study, researchers fed 10 adult mixed breed dogs intravenously for three weeks total using a similar technique as employed in the very first beagle studies. The dogs were fed a total of 132 kcal/kg/day through central venous catheters using a combination of protein hydrolysate solution, 50% dextrose solution, and an electrolyte solution containing sodium, chloride, and potassium. All canine patients maintained their weights and activity levels throughout the feeding period, proving again the viability of providing nutrients intravenously to dogs for long periods of time. In fact, the only negative findings in the study were thrombus formation in the jugular veins of five dogs and an overall great difficulty in keeping continous delivery of the nutritional admixture to such non-debilitated patients.

In contrast to human medicine's eager acceptance of parenteral nutrition, the veterinary literature of the time was very quiet aside from scattered case reports of using PN in solitary veterinary patients until a 1989 report where TPN was administered to clinically normal cats over a two week period.[10] This study attempted to replicate the canine study to determine the viability of TPN administration to cats. A total of seven clinically normal cats were given TPN containing dextrose, amino acids, soybean oil lipid emulsion, electrolytes, and vitamins. Overall, the conclusions of the study were that the patients were successfully fed via TPN. However, three cats inadvertently given more than their calculated caloric content had some negative side effects including weight gain, vomiting, depression, and oral ulceration. All cats given TPN also developed reversible normocytic normochromic anemia, hepatocellular swelling and vacuolization, and small intestinal villous atrophy. The hepatic changes were attributed to taurine deficiency in

the PN rather than the PN itself, leading to the conclusion that cats, like dogs, could be maintained for a reasonable duration of time on PN without significant permanent changes.

Part 2. Genesis of the thesis research

Moving forward into the 21st century, total parenteral nutrition has become a well-recognized feeding modality in companion animal medicine. However, despite its widespread use, there is very little information currently published regarding the use of PN admixtures in the veterinary literature. Unlike many other areas of human and veterinary medicine where much is similar between the species, allowing for significant extrapolation and translation from human to veterinary medicine, PN usage diverges between humans and animals.

To understand the basics of the current usage of PN in human and animal medicine, it is important to understand that there are two concerns when PN admixtures are compounded – nutrition and safety. It is well recognized that dextrose solutions have a long shelf life in PN admixtures and do not suffer from degradation of their nutritional value over time. Precautions such as covering amino acid solutions to protect them from light and care taken when preparing the admixtures have also virtually eliminated concerns about amino acid breakdown. [11] Multivitamins are widely known to be nutritionally the most labile of the PN components.[11,12] This is typically not a concern in veterinary medicine due to the relatively short duration that our patients are provided PN (days to weeks rather than months to years); there is simply not enough time for the average patient to develop a vitamin deficiency secondary to breakdown of the vitamin

components of PN. Additionally, care taken to cover administration bags to protect them from light as well as using specialized PN admixture bags helps to reduce chances of vitamin degradation due to light exposure as well as minimize the binding of vitamin components to the plastic PN bag (and therefore the loss of those vitamins from the admixture).[12]

The majority of safety concerns about PN admixtures have been eliminated by appropriate pharmacological preparation of solutions. The early concerns about bacterial and fungal growth in the PN solutions have proved to be unfounded because the high osmolarity and relatively low pH of PN solutions administered through central venous lines makes it very difficult for those organisms to survive.[12,13] The Maillard reaction (the decomposition of carbohydrates by amino acids such as glycine) has virtually been eliminated when these components are compounded by a pharmacist.[11,12] The precipitation of calcium and phosphorus has also been greatly reduced by proper pharmacologic preparation -- the components are added at different times during the compounding procedure to minimize chance of precipitation.[11,12]

However, the addition of lipids to PN admixtures still raises actual safety concerns regarding the duration of stability of the resulting solution. Lipid molecules have a tendency to coalesce into larger particles. The initial stages of lipid particle degradation include creaming, where lipid particle rise to the surface of an emulsion, and flocculation, where individual lipid particles become associated with each other into groupings. Both these early stages of degradation can be reversed by agitation of the solution.[11] However, once the particles coalesce into larger particles, the degradation is irreversible. In response to concerns about coalescence of lipid particles, the Food and

Drug Administration (FDA) recommends that lipid-containing admixtures be administered for no more than 24 hours.[14] This recommendation has been adopted by the University of Missouri-Columbia Veterinary Medical Teaching Hospital (VMTH) and other veterinary institutions for all lipid-containing PN admixtures.

The normal size of chylomicrons in the bloodstream is 0.4 to 1.0 μm. A lipid-containing solution is considered unsuitable for use when coalescence of lipids leads to particles larger than 5.0 μm in size and these particles make up greater than 0.4% of a solution; such an abundance of large particles puts an animal receiving PN at risk for lipid-induced pulmonary emboli.[11,13, 15,16] The early work that led to the determination of the upper limit of "safe" lipid particle size was performed in 1965.[17] The investigators found that hypotension and dyspnea were apparent in dogs when fat particles greater than 6 μm were given intravenously. The basis of the 0.4% cut-off for the quantity of large lipid particles comes from studies in 1969 and 1986 that determined clearance of lipid particles from the bloodstream by various organs. Approximately 0.4% of lipids injected into an animal were cleared by the lung.[18,19]

There have been several investigations evaluating the best technique for the direct assessment and measurement of lipid particles in solutions. The most accepted published techniques are light obscuration particle measurement, photon correlation spectroscopy (PCS), and a transmission electron microscope technique (TEM).[20-22] It has been concluded that TEM and PCS are roughly equivalently accurate techniques and superior to light obscuration particle measurement for particle size enumeration.[22] In comparing PCS to TEM, particles smaller than 100 nm are easy to identify with TEM and particles smaller than 120-150 nm may be difficult to detect using PCS.[23] A recognized potential

limitation of all particle-sizing techniques is that the sample sizes examined are very small and therefore may not be representative of the entire admixture.[24] Nevertheless, TEM imaging is considered an accurate and reliable means for identifying particle size in lipid emulsions.

As indicated above, stability studies of PN admixtures kept at room temperature for more than 24 hours are very few in number, largely due to the adherence of most institutions to the FDA recommendations, which precludes the need for such studies. However, the degradation of lipid admixtures is known to be time and temperature dependent.[14] Multiple published studies have determined convincingly that lipid admixtures can be safely stored at refrigeration temperatures for days to weeks prior to their administration to a patient over 24 hours.[25-29] The few studies that have examined lipid particle size when admixtures were kept at room temperature for more than 24 hours employed a variety of techniques to detect lipid particle size from visual inspection to light microscopy to Coulter counting.[16,30,31,32] Within the limitation of the techniques employed, these studies suggested that lipid containing admixtures were stable for greater than 24 hours.

It is a simple fact that in veterinary medicine the medical needs of the patient must be balanced with the financial ramifications for the client, especially since the majority of our veterinary patients are not covered by health insurance. As such, one of the limitations to the use of PN is its cost. A large portion of the cost of PN is due to the special bag in which the PN formulation is stored (33% of the daily cost of PN at the VMTH Pharmacy). If a bag of PN can be used for 48 hours or more, the cost-effectiveness of administering PN would be enhanced. It would also be more convenient

for hospital staff, and result in cost savings for the client, if daily bag changes were not necessary. Additionally, as reported in the human literature, the fewer changes to the PN line and handling of the catheter port used to administer PN, the less chance of mechanical complications and introduction of catheter-related infections.[11,13,33,34] Thus, if daily line and bag changes are not necessary, the chances of these complications would be reduced.

Therefore, from these beginnings came the goals of this thesis research. The first goal was a determination -- using electron microscopy -- of the duration of time before lipid particles coalescenced into pro-embolic particles at room temperature. A second goal was to determine if manipulations to the PN will prolong the lifespan of admixtures. Such manipulations included refrigeration of PN as it was administered, agitation of the admixtures to re-suspend the lipid particles in solutions, and filtering to remove any particles larger than 5 μm.

The overriding goal of the entire project was to determine how to maximize the duration that a single bag of PN could be administered to a patient before lipid particle destabilization. This determination would hopefully not only result in savings for the client by prolonging the hang-time of a single bag of PN but also limit the frequency that PN bags are changed, reducing mechanical and sterility concerns regarding changing the PN bags. The hope was that a more precise determination of the safe hang-time of PN bags would be of wide-reaching interest to those veterinarians implementing lipid-containing PN admixtures in their hospitals, and would potentially standardize care in the duration of use of those same PN admixtures.

Part 3. From here to there

In studying the history of the human mind one is impressed again and again by the fact that the growth of the mind is the widening of the range of consciousness, and that each step forward has been a most painful and laborious achievement.

Carl Jung (1875 - 1961)

History is the witness that testifies to the passing of time; it illumines reality, vitalizes memory, provides guidance in daily life and brings us tidings of antiquity.

Cicero (106 BC - 43 BC)

All things begin with the history that is written and then are shaped by current events. The same goes for this project. As detailed in chapter one, parenteral nutrition has grown from a dream into a reality in human and veterinary medicine. And, as is clearly noted in the case of lipid particles and their degradation over time, complete understanding of the individual components and the theory of their usage in PN is of paramount importance. This brings us to chapters two and three of this thesis wherein I delve deeply into the exact formulation of veterinary parenteral nutrition including the reasoning (when known) for both the components in PN and their dosages (chapter two). The natural progression from this discussion is a discourse on exactly how to create and administer PN to a patient (chapter three). And then of course chapter four's content – our findings regarding lipid particle sizing in veterinary parenteral nutrition admixtures – is presented to the reader as a culmination of all that has gone before it.

Chapter II. A Primer on Parenteral Nutrition Part 1: Uses, Indications, and Compounding

As decreased caloric and nutrient intake can complicate the course of both mild and serious illness, parenteral nutrition (PN) is an important feeding modality for patients who are unable to receive adequate enteral nutrition. Many pharmacy facilities are capable of compounding PN formulations; with proper staff training and patient monitoring, PN can potentially be performed in most veterinary practices. Part I provides information on the components of PN formulations as well as criteria for rational selection of patients to receive PN. Part II (chapter III) discusses typical PN formulations and addresses the monitoring of patients and potential complications of PN administration.

A problem commonly faced in veterinary medicine is maintaining the nutrient and caloric requirements of patients who are hyporexic, anorexic, or otherwise unable to utilize nutrition enterally. While it is true that the goal is to discover and resolve the underlying condition, allowing the patient to go without food until the condition is resolved may contribute to morbidity and prolong therapy. In most if not all cases, supplemental nutrition is beneficial to the patient.

One potential modality for the administration of nutrition is parenteral nutrition (PN). PN describes intravenous administration of nutritional products[35] and has been successfully utilized in human medicine since 1966.[1] In veterinary medicine, PN has become progressively more widely used since the 1990's although the first published veterinary study demonstrating the provision of complete intravenous nutrition to dogs

was in 1977.[9] Parenteral nutrition may be classified as total parenteral nutrition (TPN) and peripheral (or partial) parenteral nutrition (PPN).

Definitions:

- **Total Parenteral Nutrition**

In human medicine, the strict definition of total parenteral nutrition refers to the intravenous provision of the **total** nutrient needs of the patient. As practiced in veterinary medicine, TPN may not supply all nutrient needs since the specific requirements of our critical canine and feline patients have not been as thoroughly investigated as in human patients. As such, it is controversial whether TPN formulations are appropriately supplemented with the vitamins, macrominerals, and trace elements required for long-term nutritional maintenance.[36,37] However, veterinary TPN does attempt to supply all the **energy and protein** needs of a patient. Classically, veterinary TPN is administered through a central vein due to the high osmolality of the solution.

- **Peripheral and Partial Parenteral Nutrition**

The abbreviation PPN refers to either partial parenteral nutrition or peripheral parenteral nutrition. PPN solutions are less hyperosmolar than TPN solutions and as such are safely administered into peripheral veins. It is possible to formulate PPN to provide all daily energy, protein, vitamin, and mineral requirements – these solutions are referred to as *peripheral parenteral* nutritional solutions because they are supplied through a peripheral vein. However, many times the volume of PPN required per day to meet the full energy needs of the patient is excessive because PPN has a greatly reduced osmolarity versus TPN. As such, veterinary PPN is not commonly formulated to fully supply either the daily energy or vitamin and mineral requirements but rather seeks to

provide a portion of the total energy requirements (commonly 50% of the total daily energy requirements). These solutions therefore provide *partial parenteral* nutrition.[36,37] (Table 1)

Feeding patients

- **Metabolic Changes When Food Intake Is Decreased**

Patients that require nutritional support suffer from either uncomplicated starvation or stressed starvation.[36,38] Uncomplicated starvation results when an animal is deprived of food sources, but the animal is not injured or ill. To decrease their nutrient needs, starving animals (especially dogs) will commonly lower their metabolic rates and down-regulate the release of catecholamines and other stress hormones.[36] These animals have decreased insulin secretion and rely on gluconeogenesis and hepatic glycogenolysis to supply glucose to their tissues. Fatty acids are broken down to provide ketone bodies for energy and skeletal muscle, and body proteins are broken down to amino acids that in turn are used for gluconeogenesis. In cases of short-term uncomplicated starvation, providing food will reverse the metabolic change, allowing the patient to shift back to a normal metabolic rate and begin to use carbohydrates preferentially for energy. In cases of prolonged uncomplicated starvation, there is still a potential for negative side effects to ensue when animals are re-introduced to food. One reported side effect is the refeeding syndrome (a syndrome of hypophosphatemia, hypokalemia, and hypomagnesemia induced by the introduction of food to an anorexic patient) which is discussed at length in chapter III.

Stressed starvation occurs when ill or injured animals do not have adequate food intake.[36,38] Many of these patients have elevated resting metabolic rates and an increase in

protein catabolism proportional to the extent of disease.[36] The production of catecholamines and other stress hormones is upregulated, leading to increased cardiac output and systemic vascular resistance, insulin resistance, proliferation of inflammatory mediators, and a rapid onset of malnutrition.[36]

Feeding patients suffering from stressed starvation is challenging for multiple reasons.[36,38] These animals have relative insulin resistance and do not utilize exogenous carbohydrate sources as efficiently as a non-stressed animal. It is also important to provide them with sources of amino acids to help reduce protein catabolism. Moreover, it is difficult to determine the actual energy requirements for these animals because they may have increased resting metabolic rates associated with stress and disease. However, it is equally difficult to determine the exact degree of increase of the metabolic rate. And, as discussed more thoroughly below, the underlying reason for the patient's stressed state can be important in determining how they will respond to supplemental nutrition.

- **Enteral versus Parenteral Nutrition**

When deciding to provide nutritional support to a patient, enteral nutrition (ie. orally or through feeding tubes placed directly in the gastrointestinal tract) is preferable to parenteral nutrition in patients in which it is tolerable and not contraindicated (Table 2 and 3). Enteral nutrition is more physiologic than parenteral nutrition; it has been demonstrated that the gastrointestinal tract suffers from atrophic changes when it is not utilized. Histologic examination of samples of the liver and small intestine from normal cats before and after two weeks of complete nutritional support by TPN revealed swelling and vacuolization of hepatocytes and mild to moderate small intestinal villous atrophy.[10] All changes were reversed three weeks after the animals resumed normal enteral feeding.

There is also evidence of decreased muscular contraction of the gall bladder, stomach, and duodenum during TPN administration.[39] In addition to contributing to gall bladder sludging, gut stasis may lead to bacterial overgrowth and predispose the patient to bacterial translocation and sepsis.[39,40]

Parenteral nutrition should be reserved for patients unable to utilize all or part of the gastrointestinal tract to digest and absorb nutrients. When patients have been in a good nutritional status prior to acute illness (ie. the patients were not malnourished prior to their current illness), it is possible to delay the start of nutritional supplementation for a **maximum** of five days of decreased or absent nutritional intake, although it is encouraged to start sooner.[41] It is important to take historical information into account when determining the need for nutritional support because many animals may have been anorexic or undernourished for one or more days prior to admission to the veterinary hospital. On the other hand, if the patient was already under-nourished prior to its current illness, it is important to begin nutritional supplementation immediately, regardless of the number of days of complete anorexia. (Table 4)

Components of Parenteral Nutrition

Parenteral nutritional solutions are primarily comprised of carbohydrates, amino acids, electrolytes, and possibly a lipid substrate. Some formulations will also include vitamins and mineral supplementation. The carbohydrate source utilized in the majority of TPN formulations is 50% dextrose which contributes most of the osmotic pressure of the solution. Peripheral parenteral solutions contain a lesser concentration of dextrose (typically 5% dextrose) and thus have a greatly reduced osmolarity. Most PN solutions

also contain synthetic amino acid formulations that may or may not contain electrolytes. The lipid component is supplied as a commercial lipid formulation made primarily of long-chain triglycerides derived from soybean and/or safflower sources.

- **Carbohydrates in parenteral nutrition**

The goal of using dextrose and lipid-based formulations is to provide non-protein energy sources for the patient. Dextrose is normally a readily utilizable energy source that can be transported from the bloodstream directly into the cells through the actions of insulin. Once in the cell, the glucose is readily converted into adenosine triphosphate (ATP) through the actions of the Kreb's cycle.

However, in practice, there are limitations to dextrose therapy.[41] The proportion of dextrose in a PN admixture must be limited to prevent excessive osmolarity. Dextrose in excessive quantities may lead to thrombophlebitis, especially when given through peripheral veins. Many animals suffering from stressed starvation are insulin resistant and cannot completely utilize administered dextrose. Additionally, malnourished patients commonly suffer from protein malnutrition which is not addressed by dextrose administration.

- **Lipids in parenteral nutrition**

Lipids are an efficient way to deliver energy and are often incorporated into PN solutions. Lipid emulsions have several beneficial characteristics: they are isotonic, provide energy through gluconeogenesis, ketone body production, or fatty acid oxidation, and are the building blocks of cellular membranes. The addition of lipid substrates to the PN admixture reduces the volume of dextrose required to meet an animal's energy requirements and thus reduces the solution's osmolarity.

However, controversy exists regarding the inclusion of lipids in PN, especially PN that is administered to critically ill animals. Some current research suggests that lipid use and uptake (lipolysis) may be inhibited by the presence of insulin.[43] Thus, concurrent administration of dextrose and lipids may result in inefficient lipid utilization when the dextrose stimulates insulin release.[43] However, research in healthy dogs indicates that despite an increase in insulin concentrations, PN solutions with lipids as the main source of energy do provide adequate energy.[44] It is unknown if these findings apply to patients with altered metabolism during illness.

There is also a concern that in conditions of widespread inflammation (ie. sepsis), parenterally-administered lipids may amplify the inflammatory response.[43] Most commercial lipid preparations have a predominance of n-6 fatty acids including linoleic acid. Linoleic acid is a precursor for arachidonic acid which in turn is a precursor for many pro-inflammatory mediators such as thromboxanes, leukotrienes, and prostaglandins.[45,46] Although these are essential fatty acids, recent studies suggest that exogenous lipid administration could lead to deleterious effects in patients with medical conditions perpetuated by inflammation, such as sepsis.[43,45,46]

One study compared n-6 fatty acids with n-3 fatty acid preparations in human patients with sepsis.[45] This study found that n-6 fatty acids (such as linoleic acid) upregulated endotoxin-induced monocyte cytokine production during sepsis, worsening inflammation. Interestingly, those patients receiving n-3 fatty acids showed suppression of proinflammatory cytokine production by monocytes, suggesting the possible value of lipid substrates rich in n-3 fatty acids in the formulation of future parenteral nutritional solutions.[45,46]

Additionally, a retrospective study in humans showed that patients receiving parenteral lipids had decreased platelet activating factor acetylhydrolase (PAF-AH) versus patients who did not receive lipids parenterally. PAF-AH inactivates platelet-activating factor (PAF), a pro-inflammatory mediator that may be active in critically ill or stressed patients.[47] Therefore, patients receiving parenteral lipid solutions may have an increased inflammatory response as a result of higher PAF activity. Despite this, lipids are still considered a useful component of PN, as further studies are needed to determine the clinical significance of these findings.

- **Amino acids in parenteral nutrition**

Amino acids are added to PN solutions to slow muscle breakdown and in theory to help maintain the integrity of the gastrointestinal tract and prevent atrophy.[38] By sparing proteins in the muscles and other tissues, there are larger quantities of protein in the body to aid in the function of the immune system, play a role in wound healing, and improve the function of many organs.[38,48] Simply providing non-protein energy in the form of glucose or lipids will spare protein catabolism to an extent, but some form of protein or amino acid supplementation is also required.[48,49] A study comparing the nitrogen balance in healthy dogs administered electrolytes, dextrose-containing solutions, or amino acid-containing solutions demonstrated a negative nitrogen balance in all patients except those receiving amino acid supplementation.[49]

- **Other components in parenteral nutrition**

Electrolytes, vitamins, and trace minerals are added to PN solutions to provide for non-energy daily nutritional requirements. The Nutrition Advisory Group of the Department of Foods and Nutrition has published guidelines for parenteral vitamin,

electrolyte, and trace element supplementation in human patients receiving PN. Unfortunately, such guidelines are unavailable to direct veterinary PN composition, making vitamin and mineral supplementation of veterinary PN solutions variable. Components that are commonly added to veterinary PN solutions include B vitamins, vitamin D, and vitamin A; these are often found as multivitamin supplements.[10,36,37] Human formulations may also include iron, magnesium, or selenium[35] while veterinary PN mineral supplementation is less standardized.

Although long-term PN has been administered on an experimental basis to dogs, most clinical veterinary patients only receive PN for a short period of time in comparison to their human counterparts. This minimizes the development of signs of mineral or vitamin deficiencies which humans on long term supplementation are more at risk of developing.[35] Also, veterinary patients are typically placed on enteral nutrition as soon as possible (usually within seven days); enteral nutrition is adequately supplemented with vitamins and minerals.[9,36]

It is important that veterinary patients receive appropriate electrolyte supplementation while receiving PN. In some cases, amino acid solutions contain electrolytes such as sodium, chloride, magnesium, and potassium which will typically supply the patient's needs. In other cases, the amino acid solutions are not combined with electrolytes. In this situation, the patient should receive electrolyte-containing fluids through a separate intravenous catheter or a different port on the central line.[37] As presented and discussed in chapter III, any patient receiving PN should have at least daily electrolyte panels performed in order to ensure that the patients' electrolyte needs are appropriately supplemented.

Compounding PN solutions

Asepsis is extremely important in the formulation and administration of PN solutions. Lipid-containing admixtures (dextrose, amino acids, and lipids) are significantly more supportive of bacterial and fungal growth compared to dextrose/amino acid solutions alone.[50] However, with the advent of sterile formulations of lipids and sterile technique used by pharmacists when compounding PN solutions, contamination can be minimized in today's formulations.[34,51,52] To ensure the safety of PN formulations, human and veterinary pharmacies must adhere to comprehensive guidelines regarding PN compounding and formulation.[11]

Other concerns about the stability of PN formulations are minimized when PN is formulated by a pharmacist. The stability of the PN formulation is dependent upon the techniques and order in which the components are added. The Maillard reaction (aka. the browning reaction) refers to the negative interaction between amino acids (such as glycine) with carbohydrates.[11,35] The brown color of the resulting solution is due to decomposition of carbohydrates. The pharmacist must separately prepare and combine amino acids and carbohydrates to avoid this reaction. Amino acid stability is also negatively affected by light; therefore, amino acid solutions must be carefully handled to avoid exposure to light during formulation of PN solutions.[11,35] This includes keeping amino acid solutions covered both before and after they are added to the PN solution as well as while PN is administered to the patient. However, if properly prepared and stored, dextrose and amino acid solutions are stable for several months.[11,35]

Precipitation of PN components can occur. The most commonly reported precipitation reaction is between calcium and phosphorus.[11,35,53] To avoid this, calcium gluconate is used because it is the least reactive formulation of calcium available. Also, pharmacists add calcium and phosphorus separately in the compounding regimen to allow for maximal dilution of the two nutrients in the PN solution, minimizing the chance of precipitation reactions.[11,35,53] In veterinary medicine, the composition of PN formulations vary widely and may or may not contain readily precipitant components such as calcium and phosphorus.

A third compatibility issue is the stability of lipid particles.[11,35,53-55] Over time, lipid particles begin to associate together, forming a layer at the surface of the admixture. This phenomenon is known as *creaming* and can be reversed by agitation of the admixture. Creaming occurs almost immediately after the lipid-containing admixture is compounded. If particle association is unimpeded, lipid particles in the cream layer will then begin to associate together into aggregates (*flocculation*) which eventually leads to *coalescence* of lipid particles into larger particles.

It is accepted in human medicine that when lipid particles greater than 5 μm in size make up more than 0.4% of a PN solution, embolization of pulmonary capillaries can occur.[35,53,55] The exact time point of flocculation and coalescence varies depending on temperature of the solution, components of the solution, hang time of the bag of PN, and whether the bag has been agitated. However, it is believed that unacceptable amounts of coalescence does not take place until the PN solution has been kept at room temperature for at least 24 hours.

Lipid particulate association is increased with decreasing pH and when

admixtures contain more cations than anions.[11,14,35,53-56] Increased cation concentration neutralizes the negative charge on lipid particles, decreases electrostatic repulsion between lipids, and increases the likelihood of coalescence. Appropriate formulation of PN solutions by a pharmacist is the best way to minimize the chance of these reactions.

Parenteral nutrition should only be compounded in specialized bags. Parenteral nutrition bags are composed of several layers of ethylene vinyl acetate and other components to make them poorly permeable to air. Air trapped in the bag during compounding or diffusion of air into the bag can result in air bubbles that oxidize PN components as well as trigger alarms in the intravenous pumps used to administer PN.[14,53,57] Ethylene vinyl acetate is less likely to bind to components of PN (such as vitamins and lipids) which helps to protect the components of PN from oxidation reactions.[14,53]Additionally, bags not made of ethylene vinyl acetate have the potential of releasing carcinogens into the PN solution.[53]

Obtaining and Administering PN

Veterinary parenteral nutrition can be compounded at most pharmacies that prepare PN solutions for humans, since all components used are human products. Some large veterinary referral institutions will formulate and ship PN to private practices as do commercial pharmacies. Parenteral nutrition solutions can be formulated and stored for days or weeks at refrigerated temperatures (2-8ºC)**.**[14,25,29,35,52,53-55] However, once the PN formulation warms to room temperature, most institutions recommend changing PN bags daily as per FDA recommendations to avoid contamination and lipid particle destabilization.[14] There is some debate regarding the longest amount of time that a PN

bag can be administered at room temperature with some institutions administering a single bag of PN for 48 hours or more. However, there is little published data to support the safety of this deviation from FDA guidelines.[58]

Traditionally, PN solutions in veterinary medicine have been administered continuously over 24 hours, largely due to their utilization at referral institutions where 24 hour care is available. However, the calculated daily energy requirements can safely be administered over shorter periods of time without adverse effects.[50,59,60] One investigation demonstrated complete daily nutrient infusion over 10 hours in healthy dogs.[59] The shorter administration time increases convenience of administration for situations when 24 hour monitoring is not readily available. A case study describing the mistaken administration of 1800 mL of TPN solution in two hours to a German Shepherd dog (calculated hourly rate for this patient was 50 mL/hr) caused transient hyperglycemia, hyperlipidemia, and osmotic diuresis that was reversible by aggressive intravenous fluid therapy.[61] Obviously, this infusion rate exceeds recommendations, but this case study demonstrates that TPN can be given without lasting negative effects at faster rates than those during a 24 hour continuous rate infusion.

As mentioned above, veterinary TPN is administered through a central vein due to the high osmolarity of the solution. Total parenteral nutritional solutions are always hyperosmolar compared to the plasma. Normal plasma osmolarity is approximately 300 mOsm/L, while TPN solutions are typically at least 850 mOsm/L, and commonly 1500-2000 mOsm/L. Hyperosmolar solutions can directly damage the tunica intima of blood vessels. Also, red blood cells and other cells can lyse when they are exposed to a hyperosmolar environment in the bloodstream. Therefore, TPN solutions must be

administered through a central venous catheter – typically one placed into or terminating in the jugular vein – to allow for dilution to an iso-osmolar solution; this occurs when TPN quickly mixes with a relatively large volume of blood in a central vein. Early studies in Beagles proved the safety of administering hyperosmolar solutions through a central vein; hyperosmolar solutions up to 2400 mOsm/L were diluted by the bloodstream to isotonicity within 1.5 to 2.5 cm from the point of infusion into the central vein.[1]

Another significant issue in the administration of PN is catheter-related infection introduced by catheter placement or improper handling of intravenous tubing or ports. Human medicine adheres to strict guidelines to prevent such complications and most of these guidelines have been adapted to veterinary medicine.[62] (Table 5)

Minimizing manipulation of and contact with the intravenous catheter, the administration set, and the PN bag itself – including routine line changes -- will lower the risk of catheter-related infections. Human studies of 24 hour versus 72 hour PN line changes revealed a significant decrease in the incidence of nosocomial septicemia when changes were prolonged to 72 hours.[63] The authors speculated that decreased septicemia was due to the fact that the majority of contamination was introduced through the open catheter hub during IV line changes. In the case of patients receiving PN through a central catheter, multi-lumen central catheters do not have an increased risk of infection versus single lumen catheters as long as the lumen dedicated to PN is kept sterile as outlined in table 4 and is dedicated solely to PN.[33,62,64]

Summary

Parenteral nutrition is a viable nutritional choice for small animal patients who cannot receive nutrition enterally. It is possible to both obtain and administer PN in a private practice setting. Parenteral nutrition formulations should be obtained from a pharmacy where appropriate protocols are followed to safely compound the solution. However, once the PN has been formulated, special equipment is not required for administration other than an aseptically placed and maintained catheter dedicated specifically to PN. Chapter III details the formulation of a PN solution for patients as well as potential complications when administering PN.

Key Facts

1. Very few long-term studies investigating the clinical merits of parenteral nutrition administration exist in veterinary medicine; extrapolations from the human literature indicate that there is measurable improvement.
2. Although most commonly used in the subset of patients unable to tolerate enteral nutrition, parenteral nutrition is a viable option for veterinary patients who cannot meet their caloric and energy requirements through enteral feeding alone. A combination of PN and EN may be useful in this population of critical patients.
3. Practitioners interested in parenteral nutrition need to utilize a pharmacy to compound their PN solutions.

Quiz (correct answer in boldfaced type)

1. Which one of the statements below is most correct?
   a. Veterinary total parenteral nutrition (TPN) seeks to supply every nutrient need of the patient.
   b. Due to hyperosmolarity, veterinary PPN solutions are typically provided through a central vein.
   c. Veterinary PPN solutions are typically partial parenteral nutrition.
   d. a and b

2. Veterinary total parenteral nutritional solutions are ___ to the plasma and ___ to partial parenteral solutions:
   a. Hyperosmolar; hyperosmolar
   b. Hyperosmolar; hypo-osmolar
   c. Hypo-osmolar; hyperosmolar
   d. Hypo-osmolar; hypo-osmolar

3. Which statement is true about stressed starvation and uncomplicated starvation?
   a. Stressed starvation animals have insulin resistance whereas uncomplicated starvation animals have decreased insulin secretion.
   b. Stressed starvation animals have decreased insulin secretion whereas uncomplicated starvation animals have increased insulin resistance
   c. Simply feeding stressed starved animals will allow them to shift back to normal metabolism.
   d. None of the above

4. Enteral nutrition is ____ compared to parenteral nutrition.
   a. Less physiologic
   b. More physiologic
   c. Equally physiologic
   d. None of the above

5. Patients should begin receiving parenteral nutrition after __ days of decreased or absent nutritional intake?
   a. Immediately if the patient was undernourished prior to current sickness
   b. After 4-5 days of decreased or absent nutritional intake if patient appropriately nourished prior to current sickness
   c. After more than 7 days of decreased or absent nutritional intake
   d. Both a and b
   e. a, b, and c

6. Which patient would be the best candidate for parenteral nutrition?
   a. Hepatic lipidosis cat that has not been vomiting
   b. Post-op gastrointestinal resection-anastomosis

c. Prolonged pancreatitis
d. Anorexic patient that had received exploratory surgery for biopsies

7. Which one of the following components of parenteral solutions contributes the most to the osmolarity of the solution?
a. Dextrose
b. Amino acids
c. Lipids
d. Vitamin additives

8. Which of the statements is NOT a possible negative consequence of lipid administration?
a. Inhibition of lipolysis in the presence of insulin
b. Pro-inflammatory effects
c. Provision of energy via gluconeogenesis
d. There are no negative consequences of lipid administration

9. Which of the following statements is NOT a possible sequela of the formulation of parenteral nutrition?
a. Maillaird reaction
b. Precipitation of calcium and phosphorus
c. Lipid particle coalescence
d. All of the above are possible sequelae

10. Which of the below is an important procedure for parenteral nutrition administration?
a. Clean and prep the skin aseptically
b. Change the intravenous lines every 12 hours routinely
c. Disconnect the intravenous lines when the patient is removed from the cage
d. Mix all intravenous drugs in the parenteral nutrition solution prior to administration
e. All of the above are important when administering PN

Chapter III. A Primer on Parenteral Nutrition Part 2: Formulation, Monitoring, and Complications

As decreased caloric and nutrient intake can complicate the course of both mild and serious illness, parenteral nutrition (PN) is an important feeding modality for patients unable to receive adequate enteral nutrition. Many pharmacy facilities are capable of compounding PN formulations; with proper staff training and patient monitoring, PN can potentially be performed in many veterinary practices. This chapter provides practitioners with basic information on the formulation and use of PN in their patients. Information is also provided on the monitoring of patients receiving PN as well as ways to overcome and identify the common complications of animals receiving PN.

Parenteral nutrition (PN) involves the intravenous administration of nutrients. Parenteral nutrition solutions are commonly mixtures of dextrose solutions, lipid emulsions, and amino acid solutions that variably contain electrolytes, vitamins, and mineral supplements (chapter II). When dextrose, lipids, and amino acids are used together in a PN formulation, the resulting mixture is termed a "3-in-1 solution" or "total nutrient admixture." Three-in-one admixtures are easy to administer, provide for a patient's short term energy needs in one solution, and are well tolerated by patients.[35] Dextrose and amino acid solutions, without lipids, are given to patients as well. These solutions avoid any potentially negative side-effects of lipid administration, but because of their high osmolarity require substantially greater volumes of delivery to meet caloric needs (see chapter II for a full discussion of the possible drawbacks of lipid supplementation).

Published opinions differ on the appropriate estimation of nutrient requirements for a patient. For the purposes of the following discussion, we will describe the formulation method used by the University of Missouri-Columbia Veterinary Teaching Hospital and several other veterinary colleges. The formulation is based on descriptions of Remillard and Thatcher[37] and Lippert and Armstrong[66] and is similar to other methods described in the veterinary literature.[36,42,67] However, research is ongoing in the field of veterinary nutrition, and the future may hold alterations to this formulation.

Total Parenteral Nutrition Formulation

For a complete case example of formulating TPN, see tables 6 and 10.

Step I: Calculate the basal energy requirements (BER)

BER (kcal/day) = $(30 \times BW_{kg}) + 70$ for patients ≥2 kg and ≤45kg

BER (kcal/day) = $70(BW_{kg})^{0.75}$ for patients <2 kg and >45kg

The BER or basal energy requirement is a measured approximation of the energy expenditures for an animal under a set of restricted conditions including environmental temperature and animal activity level. It is typically used as an approximation for a patient's resting energy requirement (RER) which describes an animal with a minimal activity level in unrestricted conditions.[37] Often the BER and RER are used interchangeably in the literature. For the body weight range indicated, the first BER equation is a useful linear interpolation of the second equation, which is commonly known as the Kleiber-Brody equation.

A recent publication indicates that neither equation is a perfect estimation of BER for individual animals – the equations better represent the energy needs of populations of animals.[68] Nonetheless, the equations are commonly used as a starting point for BER estimation. Some sources propose using the following equation for feline energy requirements: BER = 40 × BW(kg).[58] Proponents of this equation argue that for "typical" adult cats ranging between 2 and 6 kg, the total calculated caloric intake is too great when $(30 \times BW_{kg}) + 70$ is used to estimate BER and, by extension, patients are being oversupplemented.

Step II: Determine **TER** (total energy requirement)

TER = BER multiplied by an illness factor[37]

Traditionally, illness factors range from 1.0 to 2.0. Under the current system at the University of Missouri-Columbia, a burn patient would be assigned an illness factor of 2.0 to provide for increased losses of protein and fluid through cutaneous wounds. A patient in a hypermetabolic condition such as sepsis would have an illness factor of 1.7, whereas a patient without severe trauma or body protein losses would require only BER and thus an illness factor of 1.0. These factors were initially derived from research in humans during the late 1970s and 1980s. The factors have been reported and repeated in the veterinary literature without direct verification of their use in animals, and therefore may not be valid in animals.[37]

The validity of multiplying the BER by an illness factor has recently been questioned.[58,69] It has been shown in recent human studies that during periods of illness or post-trauma, the body naturally transitions to a catabolic state. Insulin resistance occurs and transient hyperglycemia may result. In this state, regardless of the amount of dextrose infused, the body is unable to make full use of it; this contradicts a traditional mind-set of supplying large amounts of dextrose to stressed patients to meet their energy needs. More recent studies in humans have also shown that increasing protein (as amino acids in PN) above the patient's basal metabolic needs does not effectively counter protein catabolism during periods of stress or illness.[69,70] Amino acids not utilized for body protein needs will result in use of the amino acids for generation of energy and result in proportionally increased metabolic urea and ammonia production.

Therefore, the practice of multiplying the BER by a factor to supply greater amounts of energy as protein (in the form of amino acids), dextrose, and lipids may not benefit a patient and may actually be detrimental. There is agreement within the human literature that during stress and illness, a human patient's energy requirements do not increase more than 1.2 × BER.[69] Thus, the current movement in veterinary medicine is away from utilizing illness factors for all patients in energy requirement calculations. Instead, illness factors are reserved for use on a case by case to avoid oversupplementation of patients, leading to hyperglycemia, liver dysfunction, or unwanted metabolic acid and ammonia production.[58]

Step III: Determine the protein requirement[37,66]

Typically: Adult cats	6g/kg/day
Renal/hepatic disease cats	3 g/kg/day
Adult dogs	4 g/100 TER kcal/day
Renal disease dogs	1.5 g/kg/day
Extraordinary protein loss dogs	6 g/100 TER kcal/day

As discussed above and in chapter II, parenterally administered amino acids are used to replace amino acids lost in protein turnover and other biochemical pathways. It should be appreciated that during both the fed and food deprived state, amino acid catabolism is always occurring, albeit at different rates. Hence, irrespective of whether protein requirements are met by amino acids given in PN admixtures, catabolism of amino acids always makes some contribution to body energy needs.

Although energy production from amino acid catabolism is well recognized, many parenteral formulations currently utilized in veterinary medicine (including the present formulation) do not account for energy derived from admixture amino acids. Such formulations are prepared so that the animal's energy requirement is completely supplied as dextrose and lipids. The is concern that the majority of supplemented amino acids will be converted into energy, rather than be used for protein synthesis and other anabolic processes, if insufficient energy is supplied to the patient from other sources.[37] Also, excessive amino acid supplementation may lead to the excretion of nitrogenous wastes, such as urea, using the very energy derived from the supplemented dextrose and lipids.[37]

For optimal utilization of parenteral amino acids in anabolic processes, it is believed that amino acids should be given in a certain proportion with energy. For this reason, protein requirement (in amino acids) is determined per 100 kcal of TER in dogs.[37] Because variation in body weight among adult cats is considerably less than among adult dogs, the optimal protein to energy ratio in cats is suitably expressed as a ratio of grams of protein per kilogram of body weight.

A 2001 study was conducted to determine the protein requirements of parenterally-fed normal dogs using nitrogen balance methodology.[48] It examined the presently used 4 g/kg/day estimation of canine protein requirements which originated from a 1968 publication reporting research on PN in adult beagles.[6] The recent work indicates that 2.3 g/kg/day is the intravenous amino acid requirement for clinically normal dogs fed their maintenance energy requirements (roughly equivalent to 2 × BER).[48] At this time, it is unclear whether this amount of protein can be used in diseased dogs when supplying only BER, but it is possible that the protein requirement for dogs may be significantly less than the 4 to 6 g/kg/day traditionally utilized as the daily protein requirement.

A study evaluating the effects of dietary protein restriction and amino acid deficiency on canine protein metabolism in dogs fed enterally concluded that a healthy dog's typical daily nitrogen requirement is 0.41-0.55 g /($kg^{0.75}$).[71] A recent study performed on healthy adult cats fed enterally concluded that cats require 2.7 g/kg/day of crude protein to meet their needs.[72] Both studies suggest that actual protein and amino acid requirements may be lower than current recommendations in cats and dogs. However, because both of these studies involved enteral provision of nutrients, it is

difficult to know the relevance of these findings to parenteral nutrition formulation. In addition, these were healthy animals, and a direct correlation to systemically ill pets cannot be inferred.

Step IV: Determine the volume of nutrient solutions required

1. Dextrose solution

The dextrose solution most often used in TPN admixtures is 50 % (500 mg/mL) dextrose and contains 1.7 kcal/mL. Patients typically receive 40 to 60% of their energy requirements from dextrose. As hyperglycemia is a complication of PN, it may be better to provide closer to 40% of the energy requirements as dextrose to patients at risk of insulin resistance. If such an alternative formulation is used, additional energy requirements must be supplied through increasing the lipid portion of the PN solution. In order to deliver 60% of a patient's energy (TER) from dextrose, calculate the volume of solution using the following equation:

TER × 0.60 = ___kcal/day of dextrose ÷ 1.7 kcal/mL

= ___ mL of 50% dextrose per day

2. Lipid emulsion

The lipid emulsion most often used in TPN admixtures is 20% (200 mg/mL) vegetable oil lipid and contains 2.0 kcal/mL. Ten and 30% lipid emulsions are used in special cases.

Patients typically receive 40-60% of their energy requirement as lipid. For 40% of energy (TER), calculate the volume of 20% lipid emulsion using the following equation:

RER × 0.40 = ___ kcal/day of lipids ÷ 2 kcal/mL

= ____ mL of 20% lipid solution per day

3. Amino acid solution

As with dextrose solutions and lipid emulsions, amino acid solutions are available in a variety of concentrations. The most commonly used solution is 8.5% (85 mg/mL) amino acids available with and without electrolytes. The following equation is used to calculate the volume of this solution:

_____ g protein/day ÷ 85 mg/mL × 1000mg/g

= _____ mL per day

Step V: Determine the total volume of TPN solution

___ mL total of dextrose + ____mL total of lipids + ____mL total of amino acids

Divide by 24 hours to determine the mL/hr

1st day typically administer 1/3 of this rate.

2nd day typically administer 2/3 of this rate.

3rd day typically administer at the full calculated daily rate.

Most patients are gradually introduced to TPN to avoid rebound hyperglycemia and other electrolyte abnormalities from the sudden infusion of large concentrations of dextrose (see chart 1 and the discussion under Patient Monitoring). The patient may require additional intravenous crystalloid (IV) fluids through another IV port to meet daily maintenance fluid and electrolyte requirements. In patients with severe electrolyte disturbances -- such as diabetic ketoacidotic patients – the use of amino acid formulations without electrolytes may simplify case management. In such cases, electrolyte-containing solutions can be administered through a separate IV catheter to allow more controlled titration and provision for electrolyte deficits.

Step VI: Determine the daily vitamin requirements

If the patient will not be receiving enteral nutrition for more than 5 to 7 days, the clinician may want to also supplement with 0.5 mg/kg vitamin K1 subcutaneously once weekly.

Virtually all sources indicate that B vitamins should be supplemented in patients receiving TPN.[36-38,42] B vitamins are essential for utilization of the dextrose, lipid, and amino acids delivered in the PN solution, and most patients ill enough to receive PN have B vitamin deficiencies. However, the actual amount of B vitamins required by critically ill animals and the amount added to PN solutions varies widely in the veterinary literature.

The exact formulation and dose of B vitamins supplemented is also rarely mentioned. Original sources recommend that the B vitamin preparations include at least

5-7 of the "important" B vitamin types (i.e., folic acid, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine, B_{12}) but do not specify a particular preparation nor a dosage.[2] An oft-published B vitamin supplementation recommendation is to add 1 mL of B complex vitamins per 100 kcal of energy supplied in the PN admixture[36]; this amount will supply more than the minimum nutritional requirements of B vitamins for adult dogs and cats as determined by the National Research Council on the Nutritional Requirements of Dogs and Cats.[15] Bear in mind however that those requirements reflect enteral nutrition and may not be correct when nutrition is supplied parenterally.

At our institution, a vitamin B complex solution is used that contains (per mL) 12.5 mg of thiamine HCl, 12.5 mg of Niacinamide, 2 mg of riboflavin, 5 mg of d-Panthenol, and 0.2 ppm Cobalt as vitamin B_{12}. One mL of the solution per 100 kcal of TER is estimated to well exceed the dietary requirement of dogs and cats.

Partial Parenteral Nutrition Formulation[74]

If one is administering *3-in-1 admixtures of PPN* containing lipids, dextrose and amino acids, the following guidelines apply. For a complete case example, see tables 7 and 11.

1.	Step I:	Calculate TER as detailed in steps 1 and 2 above
2.	Step II:	Partial daily energy requirement (PER) = 50% × TER

3. Step III: Determine the calorie sources for the patient.

- It is recommended that a dog or cat under 10 kg receive 25% of PER as dextrose, 25% as amino acids, and 50% as lipid.
- A dog between 10 and 25 kg can receive its energy requirements equally from dextrose, amino acids, and lipids (ie. 33% of its energy requirements from dextrose, 33% from amino acid sources, and 33% from lipid sources).
- A dog over 25 kg should receive 50% of its energy requirements from dextrose, 25% from amino acid sources, and 25% from lipid sources.

This variation in PPN solution composition is an attempt to keep the total volume consistent across patients of varying weights. However, patients less than 3 kg will still receive a volume of fluid greater than their daily maintenance fluid requirements in order to fulfill their daily energy requirements. Additionally, it is interesting to note that this formulation of PPN treats the amino acids purely as energy sources which contribute to the overall PER rather than purely to support muscle anabolism as with TPN. Since patients receiving PPN are not receiving all their nutritional requirements, they should ideally simultaneously receive enteral nutrition.

4. Step IV: Determine the volume of nutrient solutions required

1. Dextrose

5% (50 g/dL) dextrose solution = 0.17 kcal/mL PER × % calories as dextrose = ___ kcal/day dextrose ÷ 0.17 kcal/mL = ___ mL/day

By using 5% dextrose rather than 50% dextrose, the resulting osmolarity of the PN solution will be much less than that of TPN and therefore make the solution safe to administer through a peripheral vein. (See chapter II for a more complete discussion of osmolarity).

2. Lipids

20% (200 g/L) lipid emulsion = 2 kcal/mL PER × % calories as lipid = ___ kcal/day lipids ÷ 2.0 kcal/mL = ___ mL/day

3. Amino Acids

8.5% (8.5 g/L) amino acid solution = 0.34 kcal/mL PER × % calories as amino acids = ___ kcal/day ÷ 0.34 kcal/mL = ___ mL/day

An alternative method used by some practitioners to reduce PPN osmolarity is to use 3.5% amino acid solutions instead of 8.5% solutions. These 3.5% solutions contain 3.5 g of protein/L and have a reduced osmolarity versus 8.5% amino acid solutions. It is recommended that other sources be consulted for exact PPN formulations using 3.5% amino acid solutions prior to their use.

5. Step V: Determine the total volume of PPN solution per day

____ mL total of dextrose + ____mL total of lipids + ____mL total of amino acids

Divide by 24 hours to determine the mL/hr

PPN is typically started at the maintenance rate immediately although it can initially be given at half of the calculated rate for the first 6-12 hours and then increased to the full calculated rate.[58] PPN solutions are much less likely to induce hyperglycemia and refeeding syndrome than TPN, and thus less importance is placed on gradually introducing the PPN solution to the patient.

6. Step VI: Add in vitamin supplements

If the patient will not be receiving enteral nutrition for more than 5-7 days, the clinician may want to supplement with 0.5 mg/kg vitamin K subcutaneously once weekly.

Most sources indicate that B vitamins should be supplemented in patients receiving PPN. However, this recommendation varies widely with each publication and, as discussed above with TPN formulations, the exact components of the B vitamin complex are not directly defined in any source. Just as with TPN, a commonly stated B vitamin supplementation is to add 1 mL of B complex vitamins per 1000 kcal of energy supplied in the PN admixture.[73] Also, as with TPN, the assumption is made that

nutritional requirements for parenteral nutrition are the same as those for enteral nutrition.

Alternative PPN sources

An alternative to the 3-in-1 admixtures with lipids, dextrose, and amino acids is the use of PPN solutions with only amino acids and/or dextrose. There are several solutions available commercially. One such formulation is a mixture of 3% amino acids, glycerol, and electrolytes (available commercially or created by mixing 300mL of 8.5% amino acid solution with 700 mL of LRS plus 5% dextrose). Such a solution can be administered continuously for longer than 24 hours because it does not contain lipids which deteriorate over time. The published rate for this solution is 40-45 mL/kg/day.[75] A downside to this preparation is that it does not attempt to meet vitamin requirements in the patient and its osmolarity is higher than a 3-in-1 PPN admixture because it does not contain iso-osmolar lipids to dilute the hyperosmolar dextrose and amino acids. However, these PPN sources are advantageous in that the practitioner can either purchase the pre-mixed solutions or easily prepare them from readily available supplies using appropriate aseptic technique.

Be aware that when PPN and TPN solutions are metabolized, the solution is broken down to release a volume of free water equivalent to the volume of solution. PPN calculations (especially for the 3-in-1 admixtures) may require the use of a larger volume of solution than is practical or safe to administer to a given patient. It is also possible that larger patients will require additional electrolyte fluid solutions to completely meet their daily requirements, especially if the patient is in need of a high rate or volume of fluids.

Also, if electrolytes are not added to PPN solutions or if the patient has many electrolyte abnormalities, the patient will require concurrent administration of electrolyte-containing fluids intravenously through another IV catheter.

Patient Monitoring

Careful monitoring of patients receiving parenteral nutrition is important to identify and rectify any metabolic abnormalities that develop during the period of feeding.[37,42,58,67] Recommendations vary, but all sources agree that vital signs (ie. temperature, pulse, respiratory rates, patient attitude) should be serially monitored every 4 to 6 hours for the first 2 to 3 days and at decreasing frequency after that time. Body weight should be measured every 12 to 24 hours.

Blood and urine glucose should be evaluated at least every 12 hours for the first 2 to 3 days for evidence of hyperglycemic complications. All sources agree that if the patient's blood glucose is elevated persistently over 200 mg/dL in a patient, steps should be taken to combat hyperglycemia.[37,58,67] Some clinicians would be more aggressive and begin to address hyperglycemia at much lower blood glucose concentrations. Steps to address hyperglycemia include initially decreasing the PN fluid administration rate and potentially administering regular insulin to bring the blood glucose back into the normal range.[37,58] Insulin can be administered by intermittent intramuscular or subcutaneous injection or given as a constant rate intravenous infusion of 1 to 2 U/kg/24 hours for dogs or at a starting dose of 1 U/cat.[58] Regular insulin is the insulin of choice to use in these situations because of its short duration of action and the ease with which the dosage can be altered. A third option to combat persistent hyperglycemia would be to reformulate the PN solution with a smaller percentage of dextrose and a greater percentage of amino

acids and/or lipids to provide for energy requirements. Retrospective studies in veterinary medicine reveal that while common and usually transient in the majority of cases, many animals suffering from hyperglycemia will require at least temporary insulin therapy.[10,41,76,77]

Some authors feel that the blood glucose monitoring regimen should be more strict in order to avoid any chance of PN-induced hyperglycemia, especially for the first days of PN supplementation.[58] (see Figure 1) Following Figure 1, if the blood glucose concentration at subsequent rechecks remains between 250 to 300 mg/dL, the PN infusion rate should be decreased to the highest rate of infusion that maintains the blood glucose concentration below 250 mg/dL. If the blood glucose concentration rises above 300 mg/dL, the PN infusion rate should be decreased and the patient may need insulin therapy.

Serum electrolytes and renal parameters are also important to monitor at least every 24 hours for the first two to three days of parenteral nutrition administration and, if no complications are seen, less regularly thereafter. Evidence of hypokalemia, hypophosphatemia or other changes consistent with the refeeding syndrome can be handled as described below (see Refeeding Syndrome section). Azotemia – especially increases in blood urea nitrogen (BUN) – may be due to excessive protein supplementation and can be addressed by decreasing the amino acid content of the PN admixture.

Some authors feel that packed cell volume (PCV) and total protein (TP) parameters should be serially monitored in patients on PN.[67] Others feel that a patient's blood should be checked at least every 12 to 24 hours for evidence of lipemia via visual

inspection of the serum and/or through examination of serial triglyceride measurements for the first 2 to 3 days and then with decreasing frequency thereafter.[37] Lipemia might indicate excessive administration of lipid sources and can be addressed by decreasing the lipid proportion of the feeding admixture.

It is clear from all sources that animals receiving PN should be closely and serially monitored to identify and allow for correction of metabolic abnormalities. Similarly, as dictated by common sense, a patient should slowly be weaned off PN sources over the course of at least 12 to 24 hours to decrease rebound hypoglycemia or other electrolyte changes that could be induced by abrupt cessation of nutritional support.[58]

Other parameters typically monitored in patients receiving PN at the University of Missouri include central venous pressure measurements (CVP) -- especially in animals receiving additional isotonic crystalloids or electrolyte solutions with their PN. This is accomplished by utilizing a multi-lumen central venous catheter in the patient – one lumen is dedicated to PN administration and the other lumen can be used for CVP monitoring. Serial CVP measurements are used to prevent volume overload while patients are receiving nutrition. Checking the patient's serum osmolarity every 24 hours will ensure that the PN solution is not causing the patient's serum to become hyperosmolar. Both increases in CVP or serum osmolarity can be addressed by decreasing the rate of PN administration or decreasing the amount of dextrose administered in the solutions. Finally, visual inspection of the catheter site should be carried out at least every 12 hours. Extravasation of PN solutions leading to local tissue inflammation and necrosis is a potential complication of PN. If extravasation is detected,

the PN catheter must be removed and replaced in another location. Ice packs and hydrotherapy can be administered to the affected region.

Disadvantages of Parenteral Nutrition

The drawbacks of parenteral nutrition can be divided into four main categories: infection, mechanical complications, cost, and metabolic complications.

Infection occurs secondary to contamination and growth of bacteria and fungi in a PN bag, nosocomial bacterial or fungal contamination during the administration of PN, or infection introduced by bacterial translocation from the patient's own body -- specifically the gastrointestinal tract or skin at the catheter site. Contamination of the PN bag during compounding and nosocomial infection introduced during administration of PN can be controlled by careful preparation of PN and aseptic handling of the IV tubing as discussed in chapter II and reviewed in Table 8. Mechanical complications include IV line breakage or kinking, patient destruction of IV lines or catheters, clogging of the IV lines, and thrombophlebitis. Careful monitoring of patients can help to minimize these occurrences and use of polypropylene catheters have been shown to decrease the incidence of thrombophlebitis.[78-80] Metabolic complications such as biochemical or electrolyte abnormalities, may be induced by the administration of PN to a patient and can include hyperglycemia, hypophosphatemia, and hypokalemia.

In a retrospective study of PPN administration in small animals, metabolic, mechanical and septic complications were reported.[41] In this study, hyperglycemia (blood or serum glucose>120 mg/dL) was the single most frequently encountered metabolic complication. Other noted complications included hyperbilirubinemia, lipemia, and

azotemia. No patients required insulin therapy, and the hyperglycemia improved within one to three days. The likelihood of metabolic complications was not found to be significantly different between cats and dogs. Mechanical complications were more common in dogs (26% of dogs vs. 9% of cats) and included occlusion of catheters, line breakage, disconnections, and thrombophlebitis. There was only a 3% reported rate of septic complications.

In two retrospective studies of TPN usage in both dogs and cats, mechanical complications were frequent.[76,77] Forty-six percent of the mixed canine and feline population described by Lippert et al[76] had mechanical complications as compared to 21% of the feline only population described by Pyle et al.[77] Hyperglycemia (>140 mg/dL[18] and >134 mg/dL[77] respectively) was another common complication experienced by 75% of all cats[76] and 47% of non-diabetic cats[77] experiencing hyperglycemia. Similarly, 46% of all dogs[76] had high blood glucose values (>140 mg/dL) while receiving TPN. Other metabolic derangements detected in animals receiving TPN were hypo- and hypernatremia, hyper- and hypokalemia, hypo- and hypercalcemia, and hypo- and hyperphosphatemia. However, the occurrence of these abnormalities was much less common than hyperglycemia – at the most 10% of Lippert et al's canine and feline populations and at the most 34% of Pyle et al's feline only population. Lipemia was noted in 46% of cats and dogs[76] and 24% of cats.[77]

Clinical signs attributable to metabolic complications were rare in both studies, although some patients did require insulin administration for persistent hyperglycemia (greater than three days duration).[76,77] Lippert et al[76] reported that 36% of hyperglycemic dogs and 67% of hyperglycemic cats required insulin therapy. Although Pyle et al[77] did

not report a percentage of cats requiring insulin, the majority of hyperglycemic cats did require insulin therapy. There was an overall low rate of septic complications with Lippert et al showing no septic complications in either cats or dogs and Pyle et al finding TPN-associated sepsis in only 5 of 84 cats.

Parenteral nutrition is not an inexpensive feeding modality. PN components are fairly inexpensive individually, but when combined into admixtures, the cost of each component is additive. Also, when formulated by a pharmacy, there is a dispensing and formulation charge to provide for costs associated with use of the laminar flow hood, the physical materials needed to compound the solution (syringes, needles, tubing, etc), and cost for the expertise and time of the pharmacist. The specialized ethylene vinyl acetate PN bag also carries a substantial cost. At the University of Missouri-Columbia Veterinary Medical Teaching Hospital, the bag itself represents 33% of the daily cost of PN. When all this is taken into account, the cost per day of PN will be upwards of $100/day for the client and, as such, may be cost prohibitive or limit the duration that PN can be provided for a patient.

The Refeeding Syndrome

The refeeding syndrome is a complication of nutritional supplementation that, although possible in our veterinary patients, is more commonly reported in humans. It is a syndrome of severe hypophosphatemia, hypokalemia, hypomagnesemia, and other electrolyte derangements that can be induced in an anorectic, malnourished patient by providing nutrient supplementation (oral, enteric, or parenteral).[81,82] Patients typically suffer from hyperglycemia as well. Hyperglycemia and concurrent glucosuria can lead to

osmotic diuresis -- resulting in sodium and water loss. However, in other cases, especially in patients fed mainly with carbohydrate sources, feeding leads to reduced sodium and water excretion and, in some cases, can lead to increases in extracellular fluid volume and eventually peripheral edema.[82]

Hypophosphatemia is the most significant feature of the refeeding syndrome in humans.[81-83] It occurs when there has been starvation-induced loss of lean muscle mass, minerals, and water. The patient's whole body phosphorus is depleted in this stage, although bloodwork typically does not reflect this. When nutrition is provided to such a patient, the presence of carbohydrates causes the release of insulin. Insulin induces an intracellular shift of phosphorus, causing clinically measurable serum hypophosphatemia. As the patient is fed, there is a conversion from catabolism to anabolism and the body begins to create cell membranes, nucleic acids, adenosine triphosphate (ATP), and 2,3-diphosphoglycerate (2,3 DPG) – all of which require phosphorus. This demand for phosphorus in turn magnifies the pre-existing hypophosphatemia.

The refeeding syndrome is a clinically recognized syndrome that typically occurs about three days after initiation of nutritional intervention.[81,82] Hypophosphatemia leads to decreased cardiac contractility through an undefined mechanism and decreased white blood cell function. A wide spectrum of neuromuscular dysfunction can occur ranging from muscular paralysis, to cranial nerve deficits and ventilatory dysfunction. These neuromuscular changes may be due to hypoxic cellular injury resulting from decreased oxygen delivery to tissues due to decreased 2,3-DPG in red blood cells. Hypoxia may also result from decreased red blood cell delivery to tissues through capillary beds as red

blood cell membranes lose their pliability when the patient is hypophosphatemic. Severe hypophosphatemia may also lead to hemolytic anemia.

Hypomagnesemia and hypokalemia can cause similar clinical signs to hypophosphatemia, including cardiac arrhythmias, weakness, seizures, and ataxia.[82,83] The functions of magnesium are not completely characterized, but seem to parallel phosphorus and potassium in cellular actions. Both hypokalemia and hypomagnesemia occur in the refeeding syndrome mainly from the increase in insulin and accompanying shifting of potassium, magnesium, and phosphorus into the cells, leading to decreased concentrations of serum potassium and magnesium.[81-83]

Patients suffering from prolonged anorexia or starvation should be gradually introduced to parenteral and enteral feeding over the course of two to three days to acclimate the body to the infusion of calories when it has been in a starvation state, in turn minimizing the chances of inducing the refeeding syndrome. The patient should be carefully monitored, including serial electrolyte and blood glucose monitoring during the first days of patient supplementation. Not every patient will undergo this syndrome, but potentially every patient is at risk.

Although recognized and reported in humans, there are few veterinary publications that explicitly address the refeeding syndrome in animals. As noted previously, both hypo- and hyperphosphatemia were reported in animals placed on TPN and PPN.[76,77] These studies had a slightly greater prevalence of hyperphosphatemic complications than hypophosphatemic, although the numbers were very small in both studies. These studies were both retrospective and did not directly address reasons for these electrolyte abnormalities. A single veterinary case study describing the refeeding

syndrome in a chronically anorexic cat was characterized by severe hypokalemia and normal phosphorus concentrations.[84]

If persistent hypophosphatemia results from the administration of PN, the patient can receive intravenous phosphorus supplementation. The recommended phosphorus dosage is 0.003 mmol/kg/hr intravenously for the first 24 hours or 0.03 mmol/kg/hr for a total of six hours.[83] Although typically not possible in animals receiving PN, hypokalemia is best treated by oral supplementation. However, it can also be addressed through the addition of potassium to the intravenous fluids that the patient is receiving (see Table 9).[85]

Magnesium is administered in patients with serum total magnesium concentrations below 1.2 mg/dL (normal range 1.7 to 2.4 mg/dL).[86] Magnesium supplementation should be administered as a 20% dilution by combining $MgSO_4$ or MgCl with 50% dextrose. This solution can be given as a constant rate infusion of 0.75-1.0 mEq/kg/day for the first day, followed with 0.3 to 0.5 mEq/kg/day of the diluted magnesium solution for an additional three to five days. As an alternative in certain patients, oral supplementation of magnesium (magnesium oxide or magnesium hydroxide supplements) may be given at a dosage of 1 to 2 mEq/kg/day.

Summary

Parenteral feeding through TPN and PPN administration provides nutrition to improve clinical outcome, but also comes with substantive cost and inherent complications. Careful monitoring of patients while they are receiving PN can help to identify and allow for correction of these complications. It is important to have a

dedicated nursing staff that will closely monitor the patient receiving PN to avoid mechanical complications. Frequent blood draws and biochemical analyses over the first 2 to 3 days of PN administration can help to identify any metabolic complications. Careful compounding and sterility when handling PN can reduce infectious complications. Overall, parenteral nutrition is a viable option for those patients who cannot receive food enterally.

Key Points

1. Patients benefit from parenteral nutrition, but they frequently suffer from at least transient hyperglycemia and may require insulin supplementation.
2. Future refinements in parenteral formulations for dogs and cats are expected. These refinements will probably come with evidence of improved clinical outcomes in dogs and cats.
3. The refeeding syndrome can be induced in a patient fed enterally or parenterally; all patients need to be monitored for this potentially (albeit rare) side effect.

Quiz (correct answer in boldface type)

1. What is the most important role of protein as amino acids in parenteral nutrition?

   a. Providing another energy form to the body.
   b. Providing a substrate for glucose metabolism.
   c. Providing a substrate for muscle anabolism.
   d. Reducing the osmolarity of the PN solution.

2. Which one of the following statements is true?

a. In a total parenteral nutritional solution, typically patients receive 40% of their energy requirements from amino acids.
b. All patients routinely receive vitamin C supplemention in parenteral nutritional solutions.
c. The parenteral nutritional line and tubing should be changed every 12 hours to preserve asepsis.
d. Begin total parenteral nutritional solution delivery at a reduced rate and adjust upwards to the full calculated administration rate over the course of 1-2 days if the patient is tolerating the solution.

3. Which one of the following sets of parameters is important to routinely monitor in <u>all</u> patients when administering parenteral nutrition?
a. Glucose, BUN, repeat patient TPR
b. Glucose, phosphorus, amylase
c. Glucose, urine specific gravity, creatinine
d. Glucose, sodium, chloride

4. What are three reported metabolic complications of parenteral nutritional administration?
a. Hyperglycemia, hyperphosphatemia, lipemia
b. Hyperglycemia, hypophosphatemia, lipemia
c. Hypoglycemia, hyperphosphatemia, hypoalbuminemia
d. Hypoglycemia, hyperphosphatemia, hyperalbuminemia

5. What are important categories of disadvantages to parenteral nutrition described in the veterinary literature?
a. Infection
b. Cost
c. Metabolic
d. All of the above

6. Which one of the following is the most commonly reported complication of parenteral nutrition in the literature?
a. Hyperglycemia
b. Mechanical complications
c. Infection introduced into the parenteral nutrition bag during compounding
d. Sepsis

7. What steps should be taken when there is extravasation of parenteral nutrition solution at the catheter site?
a. Continue to administer the parenteral nutrition, but at a lower rate.
b. Remove the catheter from the affected site and place in another site.
c. Remove the catheter from the affected site and replace proximal to the affected site.

d. None of the above

8. Which electrolyte abnormalities are noted in the classic refeeding syndrome in humans?
   a. Hyperphosphatemia, hyperkalemia, hypermagnesemia
   b. Hyperphosphatemia, hypokalemia, hypomagnesemia
   c. Hypophosphatemia, hypokalemia, hypomagnesemia
   d. Hypophosphatemia, hyperkalemia, hypomagnesemia

9. Phosphorus is utilized by the body to make:
   a. 2.3 DPG (2,3-diphosphoglycerate)
   b. ATP
   c. Cell membranes
   d. a and b
   e. a and c
   f. a, b, and c

10. Cardiac arrhythmias, weakness, seizures, and ataxia are commonly associated with:
   a. Hypomagnesemia
   b. Hypophosphatemia
   c. Hypocalcemia
   d. All of the above
   e. None of the above

Chapter IV. **Effects of temperature and handling conditions on lipid emulsion stability in centrally administered veterinary parenteral nutrition (PN) admixtures**

Parenteral nutrition is widely used in companion animal medicine, and standardized protocols for preparation of PN admixtures have improved safety of administration. However, the addition of lipids to PN admixtures still raises concerns about coalescence of lipid particles; such coalescence may result in the formation of particles that are sufficiently large to block small pulmonary blood vessels. To reduce the risk of embolism associated with such coalescence, the FDA presently recommends administration of a **single** lipid-containing PN admixture preparation at room temperature for no longer than 24 hours.[14] An informal survey of US and Canadian veterinary teaching hospitals conducted by the authors revealed that the FDA recommendation has been adopted by many institutions but that clinicians at other establishments administer PN solutions for 48 hours or longer.[a]

A lipid-containing solution is considered unsuitable for use when >0.4% of its lipid particles are larger than 5.0 μm in diameter.[11,16,24,26,30,35,53,55,87,88] The typical diameter of chylomicrons in the bloodstream is 0.4 to 1.0 μm. An abundance of large particles in the admixture puts an animal that is receiving PN at risk for development of lipid-induced pulmonary emboli, because the internal diameter of pulmonary capillaries is 4 to 9 μm.[11,16,17,19,53,62,88]

Lipid particles have a tendency to coalesce into larger particles. The initial stages of lipid particle degradation include what is termed creaming, during which lipid particles rise to the surface of an emulsion, and flocculation, during which individual lipid

particles become associated with each other in groupings. Both of these early stages of degradation can be reversed by agitation of solutions. However, once the particles coalesce into larger particles, degradation is irreversible.

Because agitation of a lipid-containing admixture reverses lipid particle creaming and flocculation, agitation should slow the progression of coalescence and thereby prolong the period during which administration of such admixtures is considered safe. Similarly, use of an appropriately selected filter during delivery of the PN admixtures should be able to successfully remove coalesced lipid particles of embolic size, which would also potentially extend the duration of use of an admixture preparation. Results of previous research indicate that PN admixtures containing lipid emulsions can be kept for as long as one week at refrigeration temperatures (4°C) prior to their safe administration over a 24-hour period to humans, suggesting that keeping a PN admixture at refrigeration temperatures during administration should likewise prolong the preparation's period of safe administration.[25,27-29,30,31]

In published retrospective studies[76,77,89] of PN usage in dogs and cats, bags of PN solutions were either administered immediately or stored for as long as five days at refrigeration temperatures prior to administration. Although it was not always specifically stated in those reports, it appeared that a single bag of PN was not administered to a patient for >48 hours. Mechanical complications (eg, catheter occlusions or dislodgements and development of thrombophlebitis) developed in 21%[77] to 46%[76] of treated animals. Hyperglycemia was another common complication ranging from 20%[89] to 75%[76] of all patients, with cats seemingly more affected than dogs. Other metabolic derangements detected in animals receiving PN were hypo- and hypernatremia, hyper-

and hypokalemia, hypo- and hypercalcemia, and hypo- and hyperphosphatemia. However, the proportions of the three study populations that developed these metabolic derangements were much less than those that developed hyperglycemia: only as many as 10% of dogs and cats in one study,[76] 34% of cats in another study,[77] and 11% of cats in a third study.[89] There was an overall low rate of septic complications; in 2 studies, septic complications were not detected in cats or dogs[76,89] and PN-associated sepsis developed in 6% of cats in another study.[77] Lipemia was evident in 46% of cats and dogs[76] and 13% to 24% of cats.[77,89] No embolic respiratory complications were noted in any veterinary studies.

The purpose of the study reported here was to determine whether veterinary PN admixtures that are kept at room temperature (23°C) can be used for more than 48 hours after preparation without development of excessive lipid particle coalescence and whether lipid particle coalescence is prevented by filtration, refrigeration, or agitation of the preparations. By use of TEM, the intent was to evaluate the change in lipid particle size distribution in bags of standard veterinary PN admixture over time at room temperature (23ºC); the goal was to determine whether prolonged duration of bag hanging (>24 hours) increased the embolic risk of PN administration due to the development of an unacceptable proportion of large lipid particles (ie, those >5 μm in diameter). We were also interested to investigate whether the duration of safe administration of an admixture could be prolonged by physical manipulations of the PN admixture. If the duration of safe administration of each lipid-containing admixture can be extended (>24 hours), the result would be cost savings for clients. At our institution, compounding fees and bag-administration set costs account for as much as 75% of the

daily cost of PN administration. Prolonged use of admixture preparations would also be convenient for clinicians and pharmacists, especially over weekends and holidays when pharmacy services may be limited or unavailable. Our hypotheses for the present study were that a standard lipid-containing veterinary PN admixture can be kept in a static position at room temperature for more than 48 hours prior to the development of an unacceptable proportion of large lipid particles (with their inherent increased risk of embolism) and that agitation, refrigeration, and filtration of an admixture preparation will delay increases in the size distribution of lipid particles that would be considered unsafe.

Materials and Methods

Admixture preparation—Fifteen 2-L bags[b] of PN admixture were prepared according to standard compounding protocols[90] by a pharmacist at the University of Missouri-Columbia Veterinary Medical Teaching Hospital. Each bag contained 525 mL of 50% dextrose (1.7 kcal/mL),[c] 453 mL of 20% lipid emulsion (2 kcal/mL),[d] 840 mL of 8.5% amino acids and electrolyte solution (1 g/11.76 mL),[e] and 5 mL of vitamin B complex.[f] The admixture volume of each bag would support the resting energy requirement of a 10 kg adult dog for 96 hours as calculated by use of the following formula: (30 X weight [kg]) + 70 kcal/day. The reported size of the emulsified fat particles in the lipid emulsion was 0.5 µm.[91] The admixture was typical of veterinary formulations, in that 50% of the non-protein metabolizable energy was available as lipid and 50% was available as dextrose (calculated osmolarity, 1,280 mosm/L).

Procedures—Each bag of PN admixture was attached to an intravenous (IV) fluid administration set and its contents were delivered by use of an IV fluid pump[g] into a beaker to simulate IV administration to a patient. The rate of administration was 16 mL/h. All PN solution delivered into the beaker was measured daily to verify the evacuation rate.

Bags of PN admixture were allocated to 1 of 5 groups; conditions of temperature and handling for the duration of the experiment differed for each group of bags. Group 1 was composed of three bags of PN admixture that were each kept in a static position at refrigeration temperature (4°C). Group 2 was composed of three bags of admixture that were each kept at room temperature (23°C) and were placed on a test tube orbital rotating device[h]; the device continuously agitated the admixtures in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]). Group 3 was composed of three bags of admixture that were each kept at room temperature and were placed on a test tube orbital rotating device[h]; the device agitated the admixtures in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]) for 5 minutes every 4 hours. Group 4 was composed of three bags of admixture that were each kept in a static position at room temperature; the admixture solution from each bag was passed through a 5-µm filter[i] placed at the approximate midpoint of the IV fluid administration set tubing. At the end of the 96-hour collection period, two 10-mL samples of PN admixture were collected at a location distal to each filter, inoculated into trypticase-soy agar, and submitted for aerobic and anaerobic bacterial culture. Group 5 (control group) was composed of three bags of PN admixture that were kept in a static position at room temperature.

Collection and assessment of PN admixture samples—A 1.0-mL sample was collected by use of aseptic technique from the most distal port on the IV fluid administration set tubing from each bag of PN admixture at 0, 24, 48, 72, and 96 hours. Time zero samples were collected immediately from the distal port of the IV tubing after the tubing was primed with PN solution and before it was placed into the IV pump. All samples were collected during a 3-week period. During each week of that period, one of the three bags of PN admixture from each group (1 through 5) was compounded and underwent experimentation (including sample collections at the time points specified).

Immediately after collection, each sample of admixture was diluted 1:10 with physiologic saline (0.9% NaCl) solution. The diluted sample was mixed 1:1 with 2% osmium tetroxide solution and allowed to stand for 15 minutes to allow fixation. The osmium oxidized unsaturated bonds of fatty acids in the lipid particles, creating a heavy metal which stained the lipid membranes black. Approximately 5 µL of the resulting mixture was placed on a 200-mesh carbon copper grid[j] and allowed to adhere to the grid for 5 minutes. Excess solution was wicked off the grid, and the grid was washed 15 to 20 times in sterile water to remove excess fixative and other precipitates that were not affixed to the grid. The grid was then air dried prior to TEM[k] examination.

Via TEM, each grid was assessed for density of lipid particles and spatial distribution of particles (ie, clear separation or clumping of particles). Particles that were closely associated with large deposits of background material were not recognized as separate particles by the image analysis software. Thus, fields that were evaluated were those with high particle density and resolution. Electron micrographs of 6 to 8 regions of each sample grid were obtained to capture images of at least 300 discrete lipid particles.

The overall goal was to obtain usable images of a total of 1,000 lipid particles from each group over the three week study period.

The TEM image negatives were digitized and analyzed by use of software[l] with image processing and analysis applications.[m] The software enumerated and measured diameters of particles in the electron micrographs. Image processing was conducted by use of standard methods.[92] Images were optimized by use of contrast adjustment prior to the application of bilevel thresholding and watershed segmentation to allow discrimination of separate lipid particles from background material. Parameter filters were applied to remove particles <100 pixels and those that were not round in shape.

Radius and roundness were determined for each particle. Features were counted and included as particles if they were of a sufficient roundness. Roundness was calculated by use of an equation as follows:

$$\text{Roundness} = 4[\text{area}]/\pi[\text{maximum diameter}]^2$$

A roundness of 1.0 indicates a perfect circle, and features with a roundness value <0.66 were not evaluated. The diameter of a particle was determined by doubling the circumscribed radius determined for a perfect circle drawn around that particle (circle extended to the maximum borders of the particle).

Statistical analysis— Particle diameters from each TEM micrograph were determined and tabulated by micrograph number (1, 2, 3, and so forth), bag number (1, 2, 3), time (0, 24, 48, 72, or 96 hours), and group (1, 2, 3, 4, or 5) on a computerized data worksheet.[n] Treatment and sampling time effects were evaluated for mean, standard deviation (SD), median, and maximum values of lipid particle counts, and diameters. Post-hoc Dunn

multiple comparison tests were used to determine significant differences in particle size among sample collection time points. At each sample collection, the significance of effect of treatment (control conditions, refrigeration, intermittent agitation, continuous agitation, or filtration) was determined by use of a Kruskal-Wallis 1-way ANOVA on ranks. The Holm-Sidak method was applied to determine whether there was a significant difference in the number of particles counted at each time point. The Fisher exact test was used to determine whether the data within groups 1 through 4 were distinct from findings for control group 5 (ie, bags of PN admixture kept at room temperature without agitation or filtration). χ^2 analysis was used to determine the probability that 0.4% of particles were >5 μm in diameter in the control samples at 96 hours.

The number of electron micrographs evaluated for each admixture sample (six to eight) and the number of admixture bags within treatment groups (three) were based on a power analyses in which β was set to 0.8. Frequency mean and variance estimates used in the power analysis were based on previously reported observations.[93-95] Statistical analyses were conducted by use of commercially available software.[o] Significance was set at a value of $p<0.05$.

Results

Among the initial (0 hour) samples collected from bags of each group, lipid particle diameter means, medians, and maximums did not differ significantly ($p=0.65$, $p=0.82$, and $p=0.63$, respectively); particles >5 μm in diameter were not observed. Of the 3,208 particles in all groups evaluated at 0 hours, the largest particle diameter was 1.50 μm. This particle was present in a control group sample, was the largest particle detected

at any sample collection time point, and was approximately 6 times as large as the mean diameter of the control particles at 0 hours (0.26 μm; **Table 12**).

The total number of particles counted in the 96-hour samples obtained from the control group admixtures was 716. If the proportion of large particles had reached 0.4%, 2 or 3 particles >5 μm in diameter should have been detected. Because no large particles were evident in control group samples collected at any time point, the proportion of large particles would have to have been <0.019%. The probability of not finding a large particle simply by random chance would be <0.1% (χ^2;$p<0.001$) if the true frequency of large particles in the control bags at 96 hours was 0.4%.

Evaluation of particles from each group at each sample collection time point was successfully completed with the exception of 3 of the 75 sample collections. In each of those instances, particles were not evaluated because of poor quality TEM images in which lipid particles either were not evident or could not be distinguished from the background material despite examination of repeated sample preparations. These poor images were derived from samples collected at 72 hours in the refrigeration group (1) during the third study week, at 72 hours in the intermittent agitation group (3) during the third study week, and at 96 hours in the filtration group (4) during the second study week. From each of those PN admixture bags, lipid particles from subsequent samples could be evaluated. The cause of the poor image quality could not be identified.

Within a group, the number of particles counted in samples collected at each time point did not differ significantly, except for samples collected from group 2 bags that underwent continuous agitation. Within this group, more particles were detected at 96 hours than at 0 hours (596 ± 108 particles vs 304 ± 272 particles; $p=0.02$).

Compared with findings at 0 hours, lipid particle mean, median, and maximum diameters for each sample collection at 24, 48, 72, and 96 hours from the control bags (group 5) did not differ significantly ($p=0.49$, $p=0.97$, and $p=0.14$, respectively); those values for subsequent samples were also not significantly different from one another (**Figure 2**). Of the 5,256 total particles in control group samples evaluated at all time points, the largest particle diameter was 1.50 μm (at 0 hours).

Within each group (1 through 4), lipid particle mean, median, and maximum diameters at 24, 48, 72, and 96 hours were not significantly different from findings at 0 hours and values did not differ significantly among sample collection time points. Mean, median, and maximum lipid particle diameters in samples from bags exposed to the experimental treatments were also not significantly different from paired observations in samples from bags of the control group at any sample collection time point (Table 12). Furthermore, at each sample collection time point, the mean, median, and maximum particle diameters among the treatment groups did not differ significantly.

Visual inspection of each lipid admixture was conducted daily when samples were collected for analysis. Regardless of other experimental conditions, all bags kept in a static position had a visible cream layer at the admixture surface by 72 hours. The PN solutions in the group 2 bags (continuously agitated) consistently had separated into distinct layers, developing a visible oil layer in the bag, the IV fluid administration set, and the cartridge loaded in the IV pump at 72 hours (Figure 3 and 4). Culture of samples of the group 4 PN solutions collected at the end of the 96-hour collection period yielded no aerobic or anaerobic bacterial growth.

Discussion

For quantitative evaluation of lipid particles in aqueous emulsions, three techniques are generally accepted: light obscuration particle measurement, photon correlation spectroscopy (PCS), and transmission electron microscopy (TEM).[93,94,96] Transmission electron microscopy and PCS are equivalently accurate techniques and are superior to light obscuration particle measurement for particle size evaluation.[94] When directly comparing the TEM and PCS techniques, particles <100 nm in diameter are more easily identified via TEM whereas particles <120 to 150 nm in diameter are difficult to detect via PCS.[23] A potential limitation of all particle-sizing techniques is that sample sizes are exceedingly small and, therefore, could be construed as not representative of the entire admixture.[24] Nevertheless, TEM imaging is considered an accurate and reliable means for identifying particle size in lipid emulsions.

For our purposes, TEM particle sizing was considered sufficient for identifying a distribution of lipid particle diameters in PN admixtures that would be associated with increased risk of embolism. Any emulsion is considered unsuitable for IV administration when the proportion of large particles (>5 μm in diameter) in an emulsion exceeds 0.4%. In the present study, initial statistical plans included categorizing particles as <5 μm in diameter versus ≥5 μm in diameter. If the proportion of large particles had reached 0.4% in the control bags at the 96-hour time point, 21 particles >5 μm in diameter would have been observed. Despite counting and sizing 5,256 particles in 15 control bag samples during the entire study period, no particles were >5 μm in diameter in any control bag sample at any time point. This result was unexpected, particularly in samples collected after the bags of admixture had been suspended and in use for 24 hours (the current recommended period of safe use of a PN preparation) and especially after 96 hours when

particle coalescence was anticipated to be the greatest. Even after 96 hours, lipid particles in admixture samples collected from the IV fluid administration sets attached to control bags appeared to present no embolic risk.

In addition to finding an absence of particles >5 μm in diameter in the control bags at any sample collection time point, there was no difference in mean lipid particle diameters in control group samples collected at 0 and 96 hours. This result was also unexpected because the lipids were anticipated to coalesce over time, causing a shift in the distribution of lipid particle diameters toward notably greater sizes. These findings support the conclusion that, over time, the standard veterinary PN solutions used in our study that underwent no special temperature or handling manipulations did not develop a lipid particle diameter distribution associated with increased risk of embolism.

Results of the present study were in contrast to those of other studies[26,97,98] of lipid particle size. In 1 study,[97] the percentage of large particles (1.6 to 25.4 μm in diameter) significantly increased between 0 and 72 hours both in samples kept at refrigeration temperatures and those kept at room temperature. That study did not include direct assessment of particle size, but relied on Coulter counter analysis of numbers and sizes of particles at various time points. In another study,[26] admixtures containing lipid emulsion that were kept at room temperature developed particles with diameters >5 μm within 30 hours, as determined via a dynamic light scatter technique. Breakdown of the admixture solutions was visible in both of those studies. Another study[98] in which PN admixtures were examined by use of a light extinction method revealed variation in particle sizes over time; with longer time intervals, greater particle sizes as well as visual precipitation of components were detected. The reason for variance of results of those studies from the

findings of the present investigation is not completely clear; however, it is important to note that in each of the previous studies, lipid particles were not directly viewed and measured to determine their diameters.

The findings of our study are not completely without precedent. By use of scanning electron microscopy, investigators examined PN admixtures that had been stored at refrigeration temperatures for 28 days followed by administration at room temperature during a two day period and found that the mean diameter of lipid particles was not significantly increased from day 0 to day 30.[31] In that study, mean initial particle diameter was 0.27 ± 0.08 μm and particle diameter on day 30 was 0.36 ± 0.11 μm; the largest particle detected was 2.74 μm. Additionally, other studies[30,25,27-29] in which PN solutions were evaluated after storage at refrigeration temperatures for as long as 28 days and then administered during a one to two day period at room temperature revealed that there were no significant changes in mean particle diameter over time nor did the proportion of lipid particles >2.0 μm in diameter exceed 0.4%. The techniques used in those studies included scanning electron microscopy, light microscopy, PCS, and computerized particle counting methods. Results of other studies of PN admixture stability[99-101] involving dynamic light scattering and light extinction methods of lipid particle examination indicated that, although there was a gradual increase in particle size over time, the proportion of large particles that could be associated with risk of embolism did not significantly increase after 30 hours at room temperature.

Variation in admixture mineral composition may account for the reported differences in lipid particle stability and may provide a reason for the long-term stability of the PN admixtures of the present study that were kept at room temperature. Typically,

the electrostatic charge (zeta potential) on the surface of lipid particles inhibits coalescence.[16] This repulsive charge is bestowed on the lipid particles by the phospholipid surfactant in the lipid emulsion. However, the presence and amount of ions in the PN admixture, especially the presence of divalent cations such as calcium^{2+}, can alter these protective electrostatic charges.[35,97,100] Additionally, phosphorus, a standard component of PN admixtures, can precipitate with calcium and result in lipid emulsion breakdown.[35,98,101] The PN admixtures used in our study did not have calcium as a component, whereas most admixtures administered to humans (including those analyzed in other studies[16,27,28,30,31,98-100]) routinely contain calcium. It is possible that calcium hastened the breakdown of PN admixtures in previous studies. An interesting future investigation would involve the addition of calcium to the admixtures used in our study to determine whether calcium induces significant changes in lipid particle size over time.

Another intriguing finding of the present study was that after 72 hours, the PN admixture in the continuously agitated bags (group 2) had separated into distinct layers within the bag and the chambers of the infusion set and IV tubing. The uppermost layer appeared to be an oil layer, indicating coalescence of lipid particles. This is similar to the finding of an investigation[97] by Bettner and Stennett, where rings of oil began forming by 48 hours and progressed to free floating oil layers by one week in both refrigerated and room temperature admixtures. In our study, no large lipid particles were detected via TEM examination in the PN admixtures in group 2 bags, despite the presence of an oil layer. Comparison of mean and maximum particle sizes over time in the continuously agitated bags and control bags did not indicate any difference in particle size distribution.

Although it is unclear why continuous agitation of group 2 bags in our study led to breakdown of the emulsion without evidence of large lipid particles in samples collected from the distal IV port of the administration sets, we support recommendations not to administer such separated emulsions to patients to avoid pulmonary embolism.[11,14,35] Agitation may have disrupted the surfactant layer in the lipid emulsion and nullified the repulsive electrostatic charges on the lipid particles, thereby promoting coalescence. Alternatively, agitation may have overwhelmed the repulsive forces of the particles, leading to collision between and eventual coalescence of lipid particles into a visible oil layer. Because of lower density, coalesced particles may have essentially floated away from the outflow of smaller more dense intact particles in solution to create a poorly mixed oil layer. If this were the case, the less-dense large particles or oil layer may have been more likely to separate from the smaller more-dense particles in the IV fluid administration set components where the admixture was held relatively static for short periods of time. Our assumption is that the smaller more-dense particles would have continued to move through the IV fluid administration set and were collected in samples from the distal port, whereas the oil layer remained behind. This explanation could be tested by mixing the contents of the bag daily before sample collection and also by collecting samples directly from the bag rather than from the IV administration set tubing and pump; those samples could then be examined via TEM to ascertain whether large particles were present.

In the present study, the creaming that developed in the PN bags in groups 1, 4, and 5 after 72 hours was expected and consistent with previous reports of changes in lipid admixtures that remained static.[11,14,16,35,55,97] Because creaming does not indicate

coalescence of lipid particles, it was expected that the lipid particles examined microscopically would not exceed the 5-µm-diameter cut-off point in any of the samples from these groups. It is widely held that gentle agitation can reblend mixtures that have a cream layer without safety concerns regarding their administration to patients.

The finding of no aerobic or anaerobic bacterial growth in cultures of samples collected from admixtures that were held static at room temperature in our study is consistent with previous reports that bacterial growth in properly prepared and handled PN admixtures is rare and usually results from patient or hospital-related contamination.[11,35,51,62] Therefore, taking into account our findings regarding bacterial contamination, microscopic lipid particle size, and the visual appearance of admixtures, we conclude that properly compounded PN admixtures of the type used in the present study that are kept static at room temperature are unlikely to develop large lipid particles with an inherent risk of embolism when administered for longer than 48 hours, without the need for other interventions.

Chapter 5. **Future directions**.

The termination of this research supports the conclusion that lipid particles in the veterinary parenteral nutrition (PN) admixtures studied do not coalesce into pro-embolic particles over a 96-hour period. Obviously, one can then conclude that parenteral nutritional admixtures formulated as described in our study do not have to be discarded within 24 hours to protect a patient from lipid-particle-induced pulmonary embolism. However, the question still remains as to how long a single veterinary PN admixture as detailed in this thesis research can be used in a patient before simply not being nutritionally viable. Additionally, it is not clear whether the addition of other components to the admixture would alter the rate of lipid particle coalescence.

As presented in the introduction to this thesis research (Chapter 1), nutritional concerns related to nutrient degradation (especially vitamins) is very important. Thus, a study conducted in a similar fashion to this original research but with routine daily PN sampling for vitamin concentrations would be a logical next step. If it could be proved that B vitamins as well as other commonly used veterinary vitamin additives to PN are viable for greater than 48 hours, one could state that it is definitively recommended to utilize the veterinary PN solutions used in the study for 48 hours or more. This would in turn alleviate any concerns related to both nutrition and safety in the admixtures.

An additional point of future research centers around the varying results found in lipid particle size with different particle sizing techniques. As discussed extensively in Chapter 4, there is a large amount of variability in the results of similar studies looking at lipid particle sizes over time. Some studies[26,97,98] documented the development of pro-

embolic lipid particle sizes over shorter time frames than in our study while other studies[27-31,99-101] had similar findings to our work.

The first logical step to explore this inconsistency more closely would be to compare lipid particle diameters in PN admixtures created as detailed in our study using different particle sizing techniques. For example, a single bag of PN at time 0 could be examined simultaneously using the transmission electron microscope (TEM) as well as light obscuration particle sizing and Coulter counting. The results could then be compared to determine if the particle sizing technique altered the particle diameters recognized and enumerated.

Two important things could come from comparing particle sizing techniques. First, it would be intriguing to see if the results noted in this original study could be reproduced by more than one particle sizing technique. If the results could not be reproduced, it would raise important questions about the validity of each particle sizing technique. If the results could be reproduced, it would strongly support the fact that there is another factor differing between studies -- more than simply the particle sizing technique -- that has led to the discrepancies between some of the published literature and our study.

The discussion section of Chapter IV details our hypothesis that it was likely the additional components added to the PN admixtures -- calcium and other charged ions – which lead to the breakdown of the surfactant layer and eventual coalescence of lipid particles in other studies. In order to evaluate this further, a logical first step would be to add calcium to the standard veterinary PN admixture used in this project and repeat the same study design using one group of PN admixture with calcium and one group without

the addition of calcium. The same sampling and sizing techniques could be applied to these two groups to determine if there were any significant differences between PN admixtures with and without calcium. The results of such a study would provide important information regarding the role of cations such as calcium in the stability of PN admixtures.

Despite the girth of information available on PN in the human medical field, close inspection still reveals gaps in this knowledge which prompt questions that need to be answered. Our research project has contributed to one such void regarding lipid particle degradation over time. Still other areas requiring more knowledge include the possible patient-related contraindications for lipid administration (pro-inflammatory conditions, sepsis, etc) and whether medium chain triglycerides may be better PN lipid sources than long chain triglycerides. The future is ripe for further exploration into the field of lipids and PN for both veterinary and human researchers.

Appendix 1. **Expanded statistical data analysis**

Summary of Statistical Analyses

1. None of the particles in any bag of any group at any time were 5µ or larger.
2. The single largest particle identified was 1.496µ (Group 5, Bag 3, Day 0).
3. The largest average particle size was 0.373µ (Group 5, Bag 2, Day 4).
4. The largest median particle size was 0.305µ (Group 5, Bag 2, Day 4).
5. The following tables contain the descriptive statistics for each bag, each day, each time:

Bag Identification*	# Particles Counted	Average Particle Size (µ)	Standard Deviation	Maximum	Minimum	Median
1,1,0	159	0.258	0.126	0.877	0.110	0.220
1,2,0	224	0.247	0.100	0.997	0.118	0.224
1,3,0	104	0.286	0.148	1.109	0.112	0.246
1,1,1	276	0.234	0.087	0.804	0.115	0.218
1,2,1	111	0.262	0.103	0.539	0.015	0.242
1,3,1	384	0.241	0.091	0.645	0.112	0.226
1,1,2	393	0.236	0.100	0.804	0.084	0.215
1,2,2	642	0.241	0.098	1.012	0.106	0.221
1,3,2	110	0.256	0.109	0.662	0.108	0.239
1,1,3	66	0.280	0.152	0.674	0.007	0.237
1,2,3	315	0.254	0.108	0.848	0.093	0.222
1,3,3	--	--	--	--	--	--
1,1,4	259	0.264	0.109	0.007	0.903	0.110
1,2,4	248	0.253	0.104	0.749	0.172	0.188
1,3,4	768	0.222	0.091	0.839	0.109	0.202

*Bag Identification: a,b,c where a = Group Number; b = Bag Number;
c = Day of Sample
Bag Identification 1,1,0 would be for Group 1; Bag 1; Day 0
Bag Identification 1,2,2 would be for Group 1; Bag 2; Day 2

Bag Identification	# Particles Counted	Average Particle Size (µ)	Standard Deviation	Maximum	Minimum	Median
2,1,0	132	0.244	0.124	1.029	0.109	0.200
2,2,0	162	0.259	0.105	0.761	0.116	0.244
2,3,0	618	0.248	0.103	0.639	0.007	0.235
2,1,1	194	0.243	0.099	0.823	0.110	0.219
2,2,1	81	0.289	0.140	1.118	0.129	0.241
2,3,1	267	0.239	0.088	0.623	0.114	0.223
2,1,2	307	0.230	0.099	0.660	0.082	0.213
2,2,2	136	0.262	0.102	0.577	0.117	0.235

2,3,2	368	0.242	0.098	0.731	0.109	0.219
2,1,3	189	0.228	0.138	0.962	0.007	0.200
2,2,3	345	0.223	0.078	0.610	0.098	0.209
2,3,3	234	0.248	0.099	0.785	0.110	0.226
2,1,4	472	0.233	0.101	0.958	0.108	0.210
2,2,4	670	0.225	0.082	0.690	0.107	0.214
2,3,4	645	0.216	0.088	1.066	0.108	0.200

4,3,4	289	0.250	0.092	0.808	0.109	0.231

Bag Identification	# Particles Counted	Average Particle Size (μ)	Standard Deviation	Maximum	Minimum	Median
3,1,0	142	0.271	0.115	1.075	0.117	0.252
3,2,0	216	0.271	0.128	0.840	0.113	0.234
3,3,0	101	0.239	0.123	0.688	0.007	0.212
3,1,1	319	0.252	0.100	0.641	0.007	0.242
3,2,1	185	0.262	0.112	0.924	0.111	0.235
3,3,1	882	0.244	0.098	1.151	0.111	0.230
3,1,2	246	0.233	0.111	0.996	0.007	0.219
3,2,2	737	0.234	0.086	0.549	0.105	0.217
3,3,2	100	0.259	0.134	0.884	0.115	0.223
3,1,3	143	0.229	0.132	0.817	0.007	0.197
3,2,3	357	0.252	0.099	0.922	0.114	0.234
3,3,3	--	--	--	--	--	--
3,1,4	307	0.243	0.099	0.821	0.106	0.222
3,2,4	98	0.282	0.117	0.636	0.128	0.256
3,3,4	144	0.262	0.103	0.635	0.102	0.241

Bag Identification	# Particles Counted	Average Particle Size (μ)	Standard Deviation	Maximum	Minimum	Median
4,1,0	157	0.241	0.081	0.533	0.118	0.233
4,2,0	84	0.275	0.125	0.751	0.025	0.263
4,3,0	150	0.248	0.110	1.053	0.118	0.227
4,1,1	1183	0.223	0.083	0.755	0.007	0.211
4,2,1	99	0.238	0.126	0.981	0.007	0.219
4,3,1	234	0.264	0.107	0.723	0.113	0.241
4,1,2	364	0.231	0.096	0.702	0.085	0.210
4,2,2	232	0.232	0.085	0.583	0.105	0.218
4,3,2	188	0.241	0.094	0.617	0.108	0.216
4,1,3	149	0.234	0.116	0.706	0.007	0.226
4,2,3	183	0.241	0.104	0.556	0.015	0.221
4,3,3	50	0.280	0.111	0.530	0.115	0.267
4,1,4	271	0.284	0.137	1.424	0.110	0.263
4,2,4	--	--	--	--	--	--

Bag Identification	# Particles Counted	Average Particle Size (μ)	Standard Deviation	Maximum	Minimum	Median
5,1,0	660	0.250	0.090	0.900	0.109	0.233
5,2,0	103	0.265	0.111	0.760	0.117	0.237
5,3,0	196	0.310	0.206	1.496	0.113	0.249
5,1,1	1455	0.215	0.081	0.648	0.021	0.204
5,2,1	352	0.264	0.102	0.710	0.114	0.241
5,3,1	210	0.272	0.123	0.956	0.112	0.242
5,1,2	44	0.271	0.141	0.837	0.117	0.230
5,2,2	563	0.253	0.094	0.754	0.109	0.235
5,3,2	175	0.243	0.127	0.692	0.114	0.251
5,1,3	44	0.271	0.140	0.837	0.117	0.226
5,2,3	563	0.253	0.094	0.754	0.109	0.235
5,3,3	175	0.279	0.126	0.691	0.113	0.251
5,1,4	485	0.243	0.100	1.088	0.111	0.226
5,2,4	10	0.373	0.241	0.934	0.148	0.305
5,3,4	221	0.255	0.102	0.768	0.110	0.233

6. Comparative Statistics (Repeated Measures Analysis of Variance or Friedman Repeated Measures Analysis of Variance on Ranks)
    a. Group 1, Comparison of particle diameter among times (0, 1, 2, 3, and 4 days):
        i. Comparing means for each time: $p = 0.495$
        ii. Comparing medians for each time: $p = 0.968$
        iii. Comparing maximums for each time: $p = 0.143$
        iv. Comparing number of particles counted for each time: $p = 0.463$

    b. Group 2, Comparison among times (0, 1, 2, 3, and 4 days):
        i. Comparing means for each time: $p = 0.148$
        ii. Comparing medians for each time: $p = .356$
        iii. Comparing maximums for each time: $p = 0.536$
        iv. Comparing number of particles counted for each time: $p = 0.024$ (Time 4 significantly greater than time 1; Multiple Comparison Test was Holm-Sidak Method)

    c. Group 3, Comparison among times (0, 1, 2, 3, and 4 days):
        i. Comparing means for each time: $p = 0.355$
        ii. Comparing medians for each time: $p = .344$
        iii. Comparing maximums for each time: $p = 0.530$
        iv. Comparing number of particles counted for each time: $p = 0.474$

    d. Group 4, Comparison among times (0, 1, 2, 3, and 4 days):
        i. Comparing means for each time: $p = 0.226$
        ii. Comparing medians for each time: $p = .125$
        iii. Comparing maximums for each time: $p = 0.109$

iv. Comparing number of particles counted for each time: p = 0.130

e. Group 5, Comparison among times (0, 1, 2, 3, and 4 days):
   i. Comparing means for each time: p = 0.910
   ii. Comparing medians for each time: p = 0.979
   iii. Comparing maximums for each time: p = 0.294
   iv. Comparing number of particles counted for each time: p = 0.668

7. Comparative Statistics (Analysis of Variance or Kruskal Wallis Analysis of Variance on Ranks)
   a. Time 0, Comparison of particle diameters among groups (1,2,3,4,5)
      i. Comparing Means for each group: p = 0.648
      ii. Comparing Medians for each group: p = 0.822
      iii. Comparing Maximum for each group: p = 0.627
      iv. Comparing number of particles counted for each group: p = 0.693

   b. Time 1, Comparison among groups (1,2,3,4,5)
      i. Comparing means for each group: p = 0.922
      ii. Comparing medians for each group: p = 0.890
      iii. Comparing maximums for each group: p = 0.681
      iv. Comparing number of particles counted for each group: p = 0.671

   c. Time 2, Comparison among groups (1,2,3,4,5)
      i. Comparing means for each group: p = 0.318
      ii. Comparing medians for each group: 0.084 (power = 0.368)
      iii. Comparing maximums for each group: p = 0.384
      iv. Comparing number of particles counted for each group: p = 0.938

   d. Time 3, comparison among groups (1,2,3,4,5)
      i. Comparing means for each group: p = 0.329
      ii. Comparing medians for each group: p = 0.443
      iii. Comparing maximums for each group: p = 0.212 (power = 0.167)
      iv. Comparing number of particles counted for each group: p = 0.540

   e. Time 4, comparison among groups (1,2,3,4,5)
      i. Comparing means for each group: p = 0.204
      ii. Comparing medians for each group: p = 0.058, power = 0.463
      iii. Comparing maximums for each group: p = 0.263 (power = 0.132)
      iv. Comparing number of particles counted for each group: p = 0.151 (power = 0.238)

Appendix 2. **Expanded methods for evaluation of lipid particles using Adobe Photoshop with the Fovea pro application**

TEM image negatives were digitized and analyzed using Adobe Photoshop[l] with the Fovea Pro application[m]. The software was used to enumerate and measure the diameters of particles in the micrographs. The image processing was conducted using a compilation of various standard methods described for image processing; the exact steps will be described in the following text.

1. The photoshop images were viewed using grayscale 8 bit.
2. Calibrate magnification was selected from the "measure global" menu. The calibration was set to micrometers for the evaluation of all micrographs.
3. The original photoshop image file was selected to include only the area with well-differentiated lipid particles. This allowed the user to delete obvious regions where the lipids were conglomerated into large masses and also allowed one to disregard the label present on the right lateral aspect of each micrograph. The resulting selected area from each micrograph was then pasted into a new photoshop file that could be manipulated freely.
4. Under the "image" menu, "adjustments" and then "levels" were selected. This allowed the user to manually manipulate the grayscale and the whiteness bars. The goal was to create the greatest contrast possible between the lipid particles and both the background and the other adjacent lipid particles while not losing any of the borders of the actual lipid particles themselves.
5. Under the "filter" menu, "threshold" and then "bilevel thresholding" was selected. This option allowed the Fovea Pro application to essentially assign the structures in

the micrograph to be black or white. Again, the application allowed for manual adjustment to ensure that all lipid particles were black and all background material was white. From this point on, all manipulations involved only the sections that were identified as black, referred to as *features*.

6. Under the "filter" menu, "watershed segmentation" was selected. This application forced the Fovea Pro application to create differentiation between the lipid particles that were found directly adjacent to each other.
7. Under the "filter" menu, "measure features" and then "reject features" was selected. This allowed rejection of all features that were clearly remnants of background material and/or improperly segmented portions of particles. We rejected any features less than 1000 μm.
8. Under the "filter" menu, "measure features" and then "accept features" was selected. The parameter selected was "shape" followed more specifically by "roundness." This directed the software to accept features that were clearly not linear or polygons.
9. Under the "filter" menu, select "measure all features." This created raw data from each feature remaining. The raw data was then imported into an Excel[n] spreadsheet.

Appendix 3. **Expanded description of radius and roundness determination for each lipid particle**

Radius and roundness was determined for each lipid particle captured on an electron micrograph. For all determinations of radius, the circumscribed radius was used in this study. The circumscribed radius is determined when a circle is drawn around the MAXIMUM dimensions of the polygon. This is in contrast to the inscribed radius which is determined from the circle drawn inside the dimensions of the polygon and which may not account for the maximum diameter of a particle. We elected to utilize the circumscribed radius in order to ensure that we were measuring the **maximum** diameter of all lipid particles. When dealing with lipid particles in this study, it was better to overestimate the size of the lipid particles rather than underestimate the size and deem a admixture safe that actually has a quantity of lipid particles present that could potentially lead to pulmonary embolism.

Features found on the electron micrographs were counted and included as lipid particles if they were of a sufficient roundness, ie. a roundness factor of greater than or equal to 0.66 (with 1.0 being a perfect circle). The exclusion point of 0.66 for the roundness measurement was determined by manual visual assessment of which particles appeared to be round lipid particles and which were not. In order to determine the validity of using 0.66 as an exclusion point, manual assessment was made of 543 features using the 0.66 cutoff (all particles with roundness values of less than 0.66 were considered to <u>not</u> be particles and those with roundness values greater than 0.66 were determined to be round lipid particles). The 543 features were evaluated from

micrographs from Group #1, time 72 hrs; group 4, time 96 hrs; group 5, time 72 hrs; group 3, time 0 hrs; group 1, time 96 hrs; and group 3, time 0 hrs.

When the features were examined, 14 features that were clearly not round lipid particles were falsely included in the "round" dataset from the 543 examined (2.6%) and 13 true round lipid particles (2.4%) were erroneously excluded from the "round" dataset using this criterion. Therefore, although the cut-off is subjective, it was deemed to be valid for our study based on the similar low rate of falsely excluded and included particles.

Appendix 4. **Expanded discussion of study limitations**

In our study, several limitations were included with the imaging methods above and beyond the small samples analyzed. First, although a great attempt was made to scan the grid and select representative samples for micrographs, the lipid particles were not always evenly distributed across the grid. Therefore, it was always a possibility that there were regions in which larger lipid particles were located that were not discovered by the investigator. When such a small percentage of total particles is significant (0.4%), even missing one or two particles larger than 5 μm could be significant. However, this limitation was uniform for every sample analyzed during the course of the entire experiment.

Secondly, great reliance was placed on the Adobe Photoshop and the Fovea Pro applications to be able to distinguish between lipid particles even when the borders were in apposition or there was a large amount of background material that partially obscured the borders of the lipid particles. In the majority of cases, the computer was able to successfully differentiate between lipid particles; however, there were still cases for each sample where adjacent particles were not separated but were lumped together by the computer applications, leading to miscounting and sizing of particles. Some of these 'multi-particles' may have resulted in erroneously large particle diameters, but in other cases they were excluded from the dataset due to their polygonal rather than round shape.

Thirdly, there were three occasions in which no lipid particles could be distinguished on the sampling grids at various time points despite repeated sample collection and grid preparations. These were group 1 at 48 hours during the 3rd sampling week; group 3 at 48 hours during the 3rd sampling week; and group 4 at 72 hours during the 2nd sampling

week. In each case, this inability to distinguish particles was due to the vast amount of background material that completely encompassed and obscured the particles. While it is possible that large lipid particles were present in those instances and simply were not being seen and counted, it seems unlikely in light of the distribution of small lipid particles seen on the following sampling day in each case. Additionally, at the same time point during the other two collection periods associated with that group, lipid particles were distinguished and were found to be much smaller than 5 μm.

However, despite these limitations, the data gathered from this study is deemed to be both valid and significant. See chapter IV for a discussion of the findings and conclusions that can be drawn from those findings.

Appendix 5. **TABLES**

Table 1. A comparison of veterinary total parenteral nutrition and partial parenteral nutrition.

Total Parenteral Nutrition (TPN)	Partial Parenteral Nutrition (PPN)
Marked hypertonicity (850-2000 mOsm/L)	Mild hypertonicity (avg. 500 – 600 mOsm/L)
Supply total patient energy needs	Usually do not supply all patient energy needs
Central venous administration required	Peripheral venous administration possible
May be deficient in total vitamin and mineral needs depending on formulation	Typically deficient in vitamin and mineral needs

Table 2. Indications for PPN

Indications for PPN[41]
1. Short term nutritional support in non-debilitated patients (no signs of malnutrition)
2. Patients in which central venous catheterization is contraindicated or impossible to perform
3. Supplemental nutrition to enteral feeding in order to provide complete nutrition to the patient

Table 3. Indications for TPN

Indications for TPN[42]
1. In patients unable to enterally absorb nutrients for more than 3-5 days, especially with apparent signs of malnutrition Examples – massive small intestinal resections, chronic/intractable vomiting, severe diarrhea
2. Severe prolonged pancreatitis when enteral feeding tubes (especially jejunostomy tubes) are not an option
3. Severe malnutrition with a non-functional gastrointestinal tract
4. Intolerance to enteral tube placement (including anesthesia to place the feeding tube) or force feeding

Table 4. Guidelines for the initiation of nutritional support.

Guidelines for the initiation of nutritional support[65]
1. Acute weight loss of 5% or chronic weight loss of 10% or more
2. Anorexia or inappetence for three or more days, especially in patients already receiving intravenous fluids.*
3. Indications of decreased protein intake such as cachexia, poor body condition, overall weakness, and non-healing wound(s)
*As discussed in the text, it is appropriate to initiate nutritional support sooner than three days, especially if other physical examination or historical parameters indicate the need to do so.

Table 5. Technical guidelines for the administration of PN.

Technical Guidelines for the Administration of Parenteral Nutrition (PN)
1. Place any intravenous (IV) line (central or peripheral) in aseptic fashion a. Clean and prepare skin as if for surgery b. Wear sterile gloves when placing and handling IV catheter, IV line, PN bag, etc. c. Keep catheter, IV tubing, PN bag sterile
2. Cover all connections between IV catheter, IV lines, and PN bag with sterile dressing to prevent bacterial or fungal contamination
3. Do not disconnect the patient from the bag of PN unless it is to attach a new bag of PN. Transport patient at all times -- including outdoors -- with IV lines and PN bag attached.
4. Do not use the IV catheter through which PN is being administered for any other solution – no drugs, fluids, etc through the PN tubing or IV ports
5. Minimize handling of the PN system!!
6. Place any IV (central or peripheral) in aseptic fashion a. Clean and prepare skin as if for surgery b. Wear sterile gloves when placing and handling IV catheter, IV line, PN bag, etc. c. Keep catheter, IV tubing, PN bag sterile
7. Cover all connections between IV catheter, IV lines, and PN bag with sterile dressing to prevent bacterial or fungal contamination
8. Do not disconnect the patient from the bag of PN unless it is to attach a new bag of PN. Transport patient at all times -- including outdoors -- with IV lines and PN bag attached.
9. Do not use the IV catheter through which PN is being administered for any other solution – no drugs, fluids, etc through the PN tubing or IV ports
10. Minimize handling of the PN system!!

Table 6. Sample calculation of TPN for a 10 kg dog that is suffering from pancreatitis.

Step I: Calculate the BER	30 (10kg) +70 = 370 kcal/day
Step II: Calculate the TER	In this case we will use an illness factor of 1.0. TER= 370 kcal/day
Step III: Determine daily protein requirement	4 g/100 TER kcal/day × 370 kcal/day = 14.8 g of protein/day
Step IV: Determine the volume of nutrient solutions required -- **Dextrose**	Patient will receive 60% of its daily energy requirement as dextrose 0.60 × TER = 0.60 × 370 kcal/day = 222 kcal/day as dextrose 222 kcal/day ÷ 1.7 kcal/mL of 50% dextrose = 130.5 mL/day (rounded to **131 mL/day) of 50% dextrose**
Lipids	Patient will receive 40% of its daily energy requirement as lipid 0.40 × TER = 0.40 × 370 kcal/day = 148 kcal/day as lipids 148 kcal/day ÷ 2 kcal/mL of 20% lipid solution = **74 mL/day of 20% lipid solution**
Amino Acids	14.8 g of protein/day ÷ 85 mg/mL of 8.5% amino acid solution = 174.1 mL/day (rounded to **174 mL/day) of amino acids**
Step V: Determine the total volume and hourly rate of TPN solution administration	131 mL + 74 mL + 174 mL = 379 mL/day of TPN solution 379 mL/day ÷ 24 hours = 15.8 mL/hour of TPN solution
Step VI: Determine the daily vitamin requirements	Vitamin K: 0.5 mg/kg ×10 kg = 5 mg vitamin K SQ given once weekly if needed Possibly supplementation with vitamin B For example:

	370 kcal/day ÷ 1 mL B complex/1000 kcal = 0.37 mL
Step VII: Administer the TPN	Day 1 – administer 1/3 of calculated requirement = 1/3 (379 mL/day + 0.37 mL of B vitamins/day) ÷ 24 hrs = 5.4 mL/hr Day 2 – administer 2/3 of calculated requirement = 2/3 (379 mL/day + 0.37 mL B vitamins/day) ÷ 24 hrs = 10.6 mL/hr Day 3+ Administer the full calculated requirement plus 0.07 mL B vitamins per day = 379 mL/day + 5 mL B vitamins/day ÷ 24 hrs = 16 mL/hr

Table 7. Sample calculation of PPN for a 10 kg dog with a gastrointestinal foreign body that is going to surgery the next day for foreign body removal and enteral feeding tube placement.

Step I: Calculate the TER	BER= 30 (10kg) +70 = 370 kcal/day In this case we will use an illness factor of 1.0. TER= 370 kcal/day
Step II: Calculate the PER	PER = TER × 50% = 370 kcal/day × 0.50 = 185 kcal/day
Step III: Determine calorie sources for this patient	A 10 kg dog will receive 1/3 of PER from protein, dextrose, and lipid sources. 185 kcal/day × 0.33 = 61.3 kcal/day Therefore, **61.3 kcal/day** from amino acids, dextrose and lipids respectively.
Step IV: Determine the volume of nutrient solutions required – **Dextrose**	61.3 kcal/day ÷ 0.17 kcal/mL of 5% dextrose = 360.5 mL/day (rounded to **361 mL/day) of 5% dextrose**
Lipids	61.3 kcal/day ÷ 2 kcal/mL of 20% lipid solution = 30.6 mL/day (rounded to **31 mL/day) of 20% lipid solution**
Amino Acids	61.3 kcal/day ÷ 0.34 kcal/mL of 8.5% amino acid solution = 180.2 mL/day (rounded to **180 mL/day) of amino acids**
Step V: Determine the total volume and hourly rate of PPN solution administration	360 mL + 31 mL + 180 mL = 571 mL/day of PPN solution (Note: This volume is greater than the daily maintenance water requirement estimated for a 10 kg dog) 676 mL/day ÷ 24 hours = 23.7 mL/hour of PPN solution
Step VI: Determine the daily vitamin requirements	Vitamin K: not needed in this patient because will be receiving enteral nutrition within the next 24 hours Vitamin B: possibly consider supplementing vitamin B For example -- 185 kcal/day (PER) ÷ 1 mL B complex/1000 kcal = 0.19 mL

Step VII: Administer the PPN	Give at full calculated rate immediately This patient will receive 24 mL/hr of PPN right away.

Table 8. A review of safety tips for administering parenteral nutrition (PN).

Tips to reduce infection:

1. Aseptic preparation of the PN solution
2. Sterile catheter placement and line handling
3. Changing/replacing intravenous lines only as needed
4. Intravenous port/line dedicated to PN
5. Small number of individuals limited to handling the PN line

Table 9. Recommended potassium supplementation for hypokalemic patients.[85]

Patient's measured K+ level	Recommended volume KCl added 1 L of fluids	Maximum rate of K+ administration
<2.0 mmol/L	80 mEq/L	6 mL/kg/hr
2.0-2.5 mmol/L	60 mEq/L	8 mL/kg/hr
2.5-3.0 mmol/L	40 mEq/L	12 mL/kg/hr
3.1-3.5 mmol/L	30 mEq/L	17 mL/kg/hr

Table 10. Blank TPN worksheet.

Step I: Calculate the BER	30 (__ kg) +70 = ____ kcal/day
Step II: Calculate the TER	In this case we will use an illness factor of 1.0. TER= illness factor × BER = _____ kcal/day
Step III: Determine daily protein requirement	Select protein requirement × _____ kcal/day = ___ g of protein/day
Step IV: Determine the volume of nutrient solutions required -- **Dextrose**	Patient will receive ____% of its daily energy requirement as dextrose _____% × TER = _____ kcal/day as dextrose _____ kcal/day dextrose ÷ 1.7 kcal/mL of 50% dextrose = _____ mL/day **of 50% dextrose**
Lipids	Patient will receive (100- ___)% of its daily energy requirement as lipid ____% × TER = _____ kcal/day as lipids _____ kcal/day ÷ 2 kcal/mL of 20% lipid solution = **____ mL/day of 20% lipid solution**
Amino Acids	____ g of protein/day ÷ 85 mg/mL of 8.5% amino acid solution = _____ mL/day **of amino acids**
Step V: Determine the total volume and hourly rate of TPN solution administration	mL of dextrose + mL of lipids + mL of amino acids = _____ mL/day of TPN solution _____ mL/day ÷ 24 hours = _____ mL/hour of TPN solution
Step VI: Determine the daily vitamin requirements	Vitamin K: 0.5 mg/kg ×_____ kg = ____ mg vitamin K SQ given once weekly if needed Possibly supplementation with vitamin B For example: BER ÷ 1 mL B complex/1000 kcal = ____ mL

Step VII: Administer the TPN	Day 1 – administer 1/3 of calculated requirement = 1/3 (____ mL/day + ____ mL of B vitamins/day) ÷ 24 hrs = _____ mL/hr Day 2 – administer 2/3 of calculated requirement = 2/3 (_____ mL/day + _____ mL B vitamins/day) ÷ 24 hrs = _____ mL/hr Day 3+ Administer the full calculated requirement plus ____ mL B vitamins per day = _____ mL/day + _____ mL B vitamins/day ÷ 24 hrs = _____ mL/hr

Table 11. Blank PPN worksheet

Step I: Calculate the TER	BER= 30 (____ kg) +70 = _____ kcal/day TER= BER × illness factor = _____ kcal/day
Step II: Calculate the PER	PER = TER × 50% = _____ kcal/day × 0.50 = _____ kcal/day
Step III: Determine calorie sources for this patient	Determine relative amounts of amino acids, dextrose and lipids supplied per day TER × proportion of dextrose = _____ kcal/day dextrose TER × proportion of amino acids = ____ kcal/day amino acids TER × proportions of lipid = _____ kcal/day lipid
Step IV: Determine the volume of nutrient solutions required – **Dextrose**	_____ kcal/day ÷ 0.17 kcal/mL of 5% dextrose = _____ mL/day **of 5% dextrose**
Lipids	____ kcal/day ÷ 2 kcal/mL of 20% lipid solution = ____ mL/day **of 20% lipid solution**
Amino Acids	____ kcal/day ÷ 0.34 kcal/mL of 8.5% amino acid solution = _____ mL/day **of amino acids**
Step V: Determine the total volume and hourly rate of PPN solution administration	mL dextrose + mL lipids + mL amino acids = ____ mL/day of PPN solution _____ mL/day ÷ 24 hours = ____ mL/hour of PPN solution
Step VI: Determine the daily vitamin requirements	Vitamin K: may or may not be needed Vitamin B: possibly consider supplementing vitamin B PER ÷ 1 mL B complex/1000 kcal = ____ mL/ day
Step VII: Administer the PPN	Give at full calculated rate immediately

Table 12—Mean ± SD and median (range) values of lipid particle diameter (determined via TEM examination at 0, 24, 48, 72, and 96 hours after preparation) in bags of PN admixture exposed to various conditions of temperature and handling (5 groups of 3 bags).

Group*	Time point (h)	No. of particles	Particle diameter (µm)	
			Mean ± SD	Median (range)
1	0	487	0.26 ± 0.12	0.24 (0.11–1.109)
	24	771	0.24 ± 0.09	0.22 (0.02–0.804)
	48	1,145	0.24 ± 0.10	0.21 (0.08–1.012)
	72	381	0.27 ± 0.13	0.24 (0.01–0.848)
	96	1,275	0.25 ± 0.10	0.25 (0.11–0.839)
2	0	912	0.25 ± 0.11	0.23 (0.01–1.03)
	24	542	0.26±0.41	0.23 (0.11–1.12)
	48	811	0.24±0.01	0.22 (0.08–0.73)
	72	768	0.23±0.01	0.21 (0.01–0.96)
	96	1,787	0.16±0.09	0.21 (0.11–1.07)
3	0	459	0.26 ± 0.12	0.23 (0.01–1.08)
	24	1,386	0.25 ± 0.11	0.23 (0.01–1.15)
	48	1,083	0.24 ± 0.11	0.22 (0.01–1.00)
	72	500	0.24 ± 0.11	0.21 (0.01–0.92)
	96	549	0.26 ± 0.11	0.24 (0.10–0.82)
4	0	391	0.25 ± 0.10	0.24 (0.02–1.053)
	24	1,516	0.24 ± 0.10	0.23 (0.01–0.981)
	48	784	0.23 ± 0.09	0.24 (0.08–0.702)
	72	382	0.25 ± 0.11	0.24 (0.01–0.706)
	96	560	0.27 ± 0.11	0.25 (0.11–1.424)
5	0	959	0.27 ± 0.13	0.23 (0.11–1.50)
	24	2,017	0.25 ± 0.10	0.28 (0.02–0.96)
	48	782	0.25 ± 0.12	0.22 (0.11–0.84)
	72	782	0.27 ± 0.12	0.23 (0.11–0.84)
	96	716	0.29 ± 0.15	0.17 (0.11–0.93)

*Bags of PN admixture were exposed to various experimental conditions; during the exposures, admixture samples were emptied from each bag via an IV fluid administration set to mimic administration to a patient. For 96 hours, three bags of PN admixture were kept in a static position at refrigeration temperature (4°C; group 1); kept at room temperature (23°C) and continuously agitated on a test tube orbital rotating device in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]; group 2); kept at room temperature and agitated intermittently on a test tube orbital rotating device in a circular motion (rotation speed, approx 360°/s [60 revolutions/min]) for 5 minutes every 4 hours; (group 3); kept in a static position at room temperature (solution passed through a 5-µm filter placed at the approximate midpoint of the IV fluid administration set tubing; group 4); or kept in a static position at room temperature without further intervention (control; group 5).

Appendix 6**. FIGURES**

Figure 1. Strict blood glucose monitoring regimen.

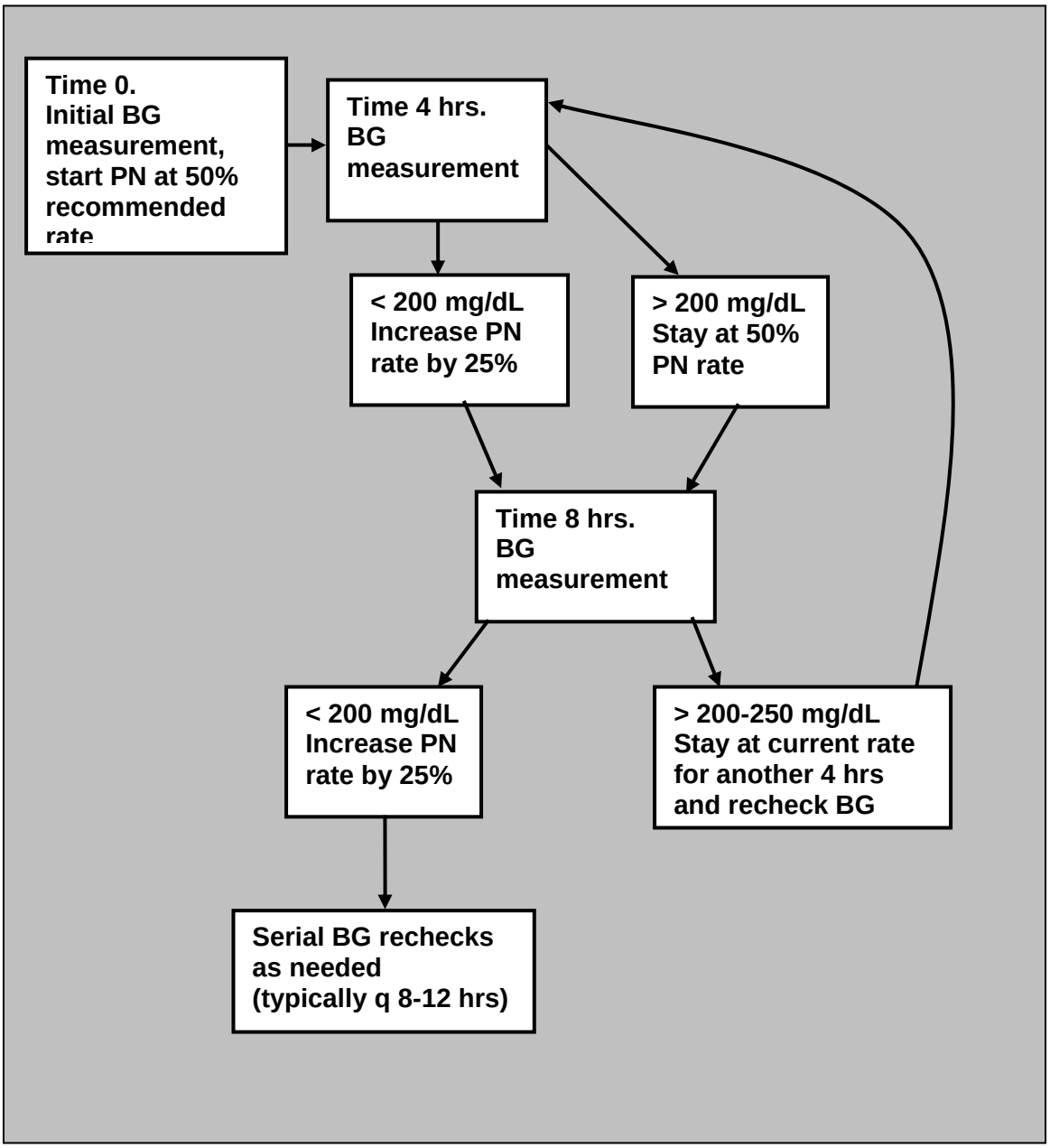


Figure 2—Mean ± SD percentage distribution of lipid particle diameters (determined via TEM examination at 0, 24, 48, 72, and 96 hours after preparation) in bags of PN admixture that were kept static at room temperature (approx 23°C; control group) for 96 hours.

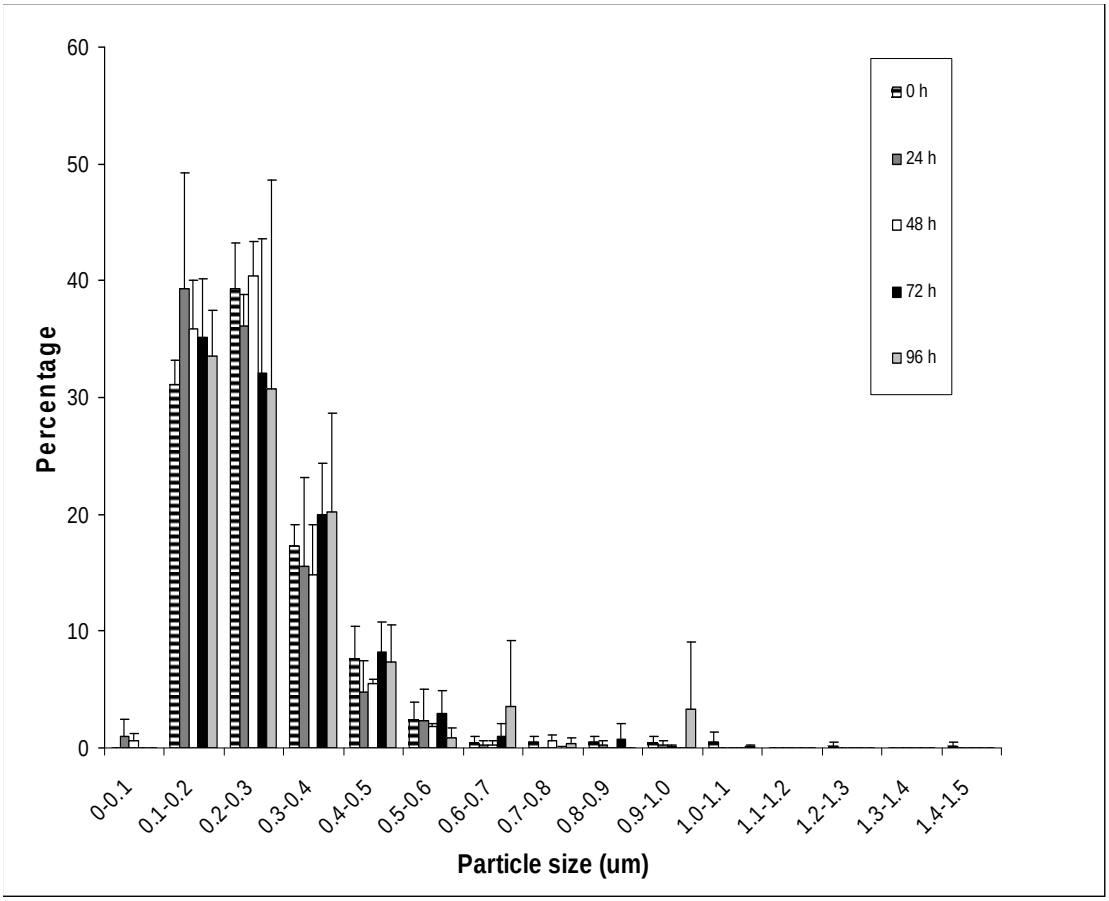


Figure 3. Oil layer in Group 2 venoset at 72 hours (continuously agitated group). Arrow indicates the oil layer

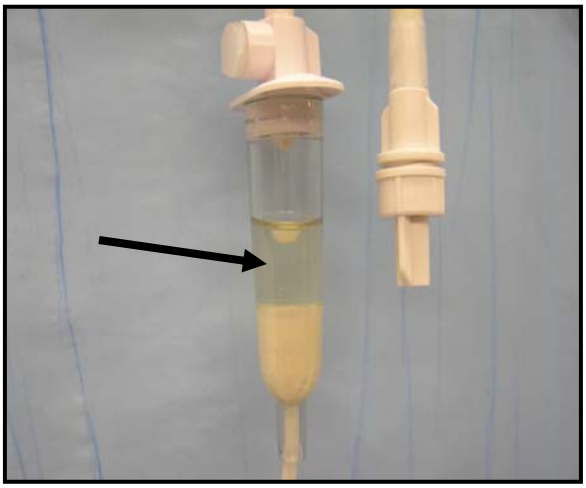
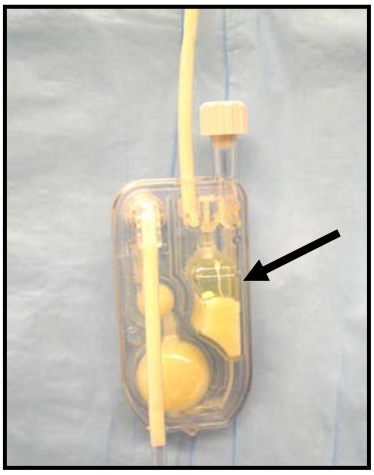


Figure 4. Oil layer in Group 2 intravenous pump cartridge at 72 hours (continuously agitated group). Arrow indicates the oil layer.



FOOTNOTES

a. Elizabeth Thomovsky, University of Missouri-Columbia College of Veterinary Medicine, Personal communication, 2005.

a. 3-in-1 mixing container, Abbott Laboratories, North Chicago, IL.

b. 50% dextrose injection, Baxter Healthcare Corp, Deerfield, IL.

c. Intralipid 20%, Baxter Healthcare Corp, Deerfield, IL.

d. Aminosyn 8.5% sulfite-free crystalline amino acid solution (Na^+, 78 mEq/L; Cl^-, 86 mEq/L; K^+, 66 mEq/L; Mg^{2+}, 10 mEq/L; acetate,61 mEq/L; and $PO4^-$, 30 mEq/L), Abbott Laboratories, North Chicago, IL.

e. Vitamin B injection solution, Phoenix Pharmaceutica Inc, St. Joseph, Mo.

f. Abbott Plum A+ infusion intravenous IV pump system, Abbott Laboratories, Abbott Park, Ill (used in groups 2 through 5) and Heska Vet/IV 2.2, Heska Corp, Fort Collins, CO (used in group 1).

g. Lab-Line lab rotator (31 X 31 cm), Barnstead International Products, Dubuque, IA.

h. Supor sterile syringe filter, Baxa Corp, Englewood, CO.

i. Carbon support film on specimen grid on 200 mesh copper, Electron Microscopy Sciences, Hatfield, PA.

k. TEM-JEOL 1200 EX, Japan Electron Optics Limited, Tokyo, Japan.

l. Adobe Photoshop, version CS2, Adobe Systems Inc, San Jose, Calif.

m. Fovea Pro 4.0, Reindeer Graphics, Asheville, NC.

n. Microsoft Excel, Microsoft Corp, Redmond, WA.

o. SigmaStat, version 3.0, SPSS Inc, Chicago, IL.

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